THE FATE OF THE UNFERTILIZED EGG IN THE WHITE MOUSE

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It has long been known that in mammals many more eggs are ovulated than develop into embryos, due in part to the failure of the spermatozoa to reach them, and perhaps more often to the inability of the eggs to implant themselves in the uterus. Such eggs are said to degenerate, but the story of their fate has been recorded in only a few instances. Since ovulation in the mouse is independent of copulation, this animal was selected for the investigation. It is natural to suppose that the process would be similar to the degeneration of the ovarian egg which has been prevented from leaving the ovary, the process termed follicular atresia.

The atresia of ovarian eggs in mammals has been described in detail by many investigators, but the degeneration of the unfertilized egg in the Fallopian tube and uterus has been given but little special study. Heape ('05) working on the domestic rabbit, mentions the finding of eggs in the tube which for some reason or other had not been fertilized and were in a process of degeneration. Hartman ('16), in his study of the early development of the American opossum, also refers to the great number of unfertilized eggs, approximately 50 per cent., which he found in the uterus, in an earlier or later stage of degeneration. He ventures the opinion that these eggs would eventually leave the uterus.

Students of atresia have usually recognized that there is an early disturbance of the nucleo-cytoplasmic relationship, although they have not always given it that name, and that it was followed by a rearrangement of the cytoplasm. Some writers however insist that a true parthenogenetic cleavage may take place, and continue in a more or less normal manner up to, in some cases,

the ten or twelve cell stage before the degenerative processes get the upper hand.

The purpose of the present investigation is to describe step by step the degeneration, whether it be fragmentation or parthenogenetic development, of the unfertilized egg in the white mouse, and to compare the process with follicular atresia in the mouse and other mammals.

The work was undertaken at the suggestion of Prof. W. R. Coe, and done under his supervision. I am pleased to acknowledge his many valuable suggestions during the course of the investigation.

Literature.

Kingery ('14) describes the method of atresia in the follicles of young mice. In his opinion the process is one of degenerative fragmentation only, that the spindle fibers of the second maturation spindle break, forming aster-like radiations and that later the achromatic fibers entirely disappear. Then the loose chromosomes form vesicles, and occasionally these fuse together to form larger vesicles. This disturbs the nucleo-cytoplasmic relationship, and an attempt to counter balance is made by the cytoplasm breaking up and surrounding the nuclei, the larger amount of cytoplasm enclosing the larger nuclear vesicles. In some cases the cytoplasmic fragments have no nucleus whatever. These fragments with or without nuclei are later absorbed, probably by the action of phagocytic cells of follicular origin.

Newman ('13) found that in the armadillo the first maturation spindle was formed in the ovary and that the egg then waited for ovulation. Eggs not located near the periphery of the ovary were not ovulated. In such cases Newman found that only a small percentage cut off the first polar body and that only three per cent. in several hundred cases gave off the second polar body, while ninety per cent. of the eggs were struck by a process of follicular atresia, which is either a cytolysis or a parthenogenetic development. He considers only the latter contingency. In such eggs there is first a casting out of the deutoplasmic mass from the formative cytoplasm. This he considers to be an act of rejuvenation on the part of the dying cell. Released from the burden of the yolk the cell is better

able to carry on the natural processes. The abstrictions of the yolk often look like multicellular structures, but Newman thinks this appearance is due to fixation. Some of these yolk fragments which he calls cytoids contain deutoplasmic granules.

Newman finds numerous tri- and multipolar spindles. These, he thinks, would result in nests of nuclei without division of the cytoplasm. Sometimes instead of a single resting nucleus formed from the maturation spindle, two nuclei might be formed instead, without the extrusion of the polar body, and suggests that from these double nuclei, the multipolar spindles might be formed. As the number of chromosomes is more than the haploid number such spindles cannot be maturation spindles. In those cells with a true cleavage spindle, Newman is inclined to the view that no polar bodies have been given off. The process is in his opinion a true parthenogenesis, but development does not proceed beyond the eight cell stage and even at that time advancing degenerative processes are to be seen.

Heape ('05) found that in the domestic rabbit copulation acted as a stimulus to certain internal rearrangements which ended in severing the ovum from its source of nourishment. At about nine hours after copulation the two polar bodies are formed and ovulation takes place about an hour later. He says: "Once freed from the ovary the mature ovum is incapable of assimilating nutriment unless it be fertilized; if from any cause fertilization is not effected, the ovum quickly dies, although it is bathed in the nutrient material supplied by the maternal tissues; ova thus degenerating are from time to time to be seen in the Fallopian tubes." It is therefore necessary for spermatozoa to be at the top of the Fallopian tube because, unlike the condition in the mouse, the ovum is dehisced from the ovary without any discus cells to provide it with food.

Bonnet ('00) reviews the different theories of degeneration and takes the view that the various spindles figured by the exponents of the parthenogenetic idea are to be considered more or less abnormal maturation spindles, and not cleavage spindles.

Van der Stricht ('01), in his study of follicular atresia in the bat, comes to the conclusion that the oöcyte of the second order forms a cleavage spindle and divides parthenogenetically. He

has traced an apparent normal cleavage as far as the six-cell stage. He also described multipolar spindles and occasionally two spindles in the same egg. The first polar body is occasionally seen to divide.

Kirkham ('07) gives an excellent review of the literature on the early development of mammalian eggs previous to 1907. The common occurrence of spindles in the polar body led him to conclude that the polar body would divide mitotically. Abnormal eggs containing tripolar spindles and in one case an egg containing two spindles were observed. Kirkham agrees with Sobotta ('95) and Rubaschkin ('06) that an egg never develops after the formation of the first polar body and the second polar spindle unless fertilization takes place, the egg degenerating within the ovary or in the Fallopian tube.

Athias ('09) also takes the view that the process is entirely one of degenerative fragmentation.

Material and Methods.

The mouse is an animal well adapted to such a study, for we know from the investigations of Sobotta, Kirkham, and later of Mark and Long, that ovulation takes place without the necessity of copulation in from thirteen and three-quarter to twenty-eight and one-half hours (Mark and Long) after parturition. The sexes were kept together in suitable cages and the females examined frequently for signs of pregnancy. Whenever a female was found to be pregnant she was placed in a cage and examined each morning. As soon as a litter was found it was removed, and a record of the time made. It is from this time record that all the ages for the various eggs are figured.

Animals were killed at intervals varying from one and one half days to four days and nine hours after the finding of the litter. The body was immediately cut open and the ovaries with tube and uterus removed and placed in the fixing solution. For this purpose Zenker with acetic, and Carnoy's 6–3–1 solution, were found to be about equally good. For tube eggs strong Flemming proved excellent. The prepared sections were stained in Heidenhain's iron hæmatoxylin, Ehrlich's hæmatoxylin, or Flemming's Triple stain, usually with suitable counter stain.

Observations.

Thirty-six hours after finding the litter, eggs are almost certain to be found in the upper part of the Fallopian tube. They always show the second maturation spindle and may or may not have the polar body attached. If present, the polar body has the chromosomes arranged in the form of a spindle (Fig. 1.) During the next twelve hours there is a change in the polar bodies. The chromatin of the spindle breaks down and arranges itself into one, two or many resting nuclei. The formation of two nuclei is well illustrated in Fig. 2. Here the vesicles have been formed but a number of chromosomes or chromatin bodies have not yet entirely lost their individuality. To one side of the polar body a small body, the first indication of a mitochondrial substance, may be seen. Fig. 3 shows a later stage in which the chromatin of the polar body has formed eight vesicle-like nuclei. Since eggs with three or more polar bodies are not infrequent (see Fig. 4), one is led to believe that the cytoplasm segments, forming itself around the various vesicles. Long and Mark ('11) find that the first polar cell often divides amitotically. They consider that this aids the degeneration and absorbtion of the polar bodies due to the increase in the exposed surfaces. Kirkham also, in work which has never been published, has observed that the first polar cell occasionally divides forming a number of fragments.

The achromatic fibers of the second maturation spindle disappear early, since in only a few cases have they been noted in eggs as old as forty-eight hours. The spindle stands out clear and distinct with the chromosomes in their natural relations but no signs of fibers can be observed.

Between forty-eight and seventy-two hours a renewal of activity begins in the unfertilized egg. Its effect is first apparent in the second maturation spindle, which may break down and form a single resting nucleus. Three eggs from a seventy-six hour mouse all have a single nucleus both in the eggs and in their polar bodies. More often however numerous vesicles instead of one result from the breaking down of the spindle. An examination of Fig. 5 will make it clear how this takes place. This egg is from a mouse in which one of the uterine horns was

closed by a tumor, thus preventing access of the sperm to the eggs on that side. The mouse was killed sixty hours after finding the litter. In the tube on the occluded side, two eggs were found, one in the second maturation spindle stage, and the other showing the breaking down of the spindle. In the other horn fertilization had taken place and the eggs were in the four-cell stage. In the figure it will be noted that the chromatin of the spindle has for the most part gone into the formation of a circular vesicle, in which a number of chromosomes remain practically unaltered.

A later stage picturing the breaking down of the spindle is that of Fig. 6. Here the lobes are larger and have become separated from the central vesicle. All the chromatin is now found in the walls of the various vesicles, none remaining as chromosomes.

The loss of the achromatic fibers and the breaking down of the spindle is probably due, as suggested by Kingery ('13), to the degenerative changes appearing quite early in the cytoplasm. This is shown by the fact that the cytoplasm stains more deeply than in normal eggs. Numerous dark staining granules are seen, particularly near the periphery of the egg. These constitute the mitochondrial bodies.

Kingery ('13) is of the opinion that the spindle fibers of the second maturation spindle break and free the chromosomes, which then migrate into the cytoplasm and form numerous nuclear vesicles. In my material the achromatic fibers disappear early, and from Figs. 5 and 6 it would appear that the vesicles may be formed *in situ*, and not necessarily from chromosomes which have wandered from the place of the original spindle.

In Figs. 7, 8 and 9 a different process of spindle disintegration is seen. These three eggs are all from the same mouse and were located close together in the lowest portion of the tube very near the uterus. From Fig. 7, it is evident that while the spindle fibers have disappeared; the chromosomes, although contracted and hugged closely together, still retain the form of a second spindle. Several chromatic fragments have evidently broken off and are seen scattered in the cytoplasm. The succeeding stage may be noted in Fig. 8. The individual chromosomes

have separated from their spindle position and lie scattered in the cytoplasm. One of the chromosomes shows the beginning of vesicle formation, which is seen completed in Fig. 9. Fig. 11 shows essentially the same condition. The polar body has divided and several small phagocytes may be seen just inside the zona. In Fig. 10 the chromatin is arranged around the periphery of the vesicle and not clumped to one side as in Fig. 9. One notices quite a similarity between these vesicles and those in process of formation in Fig. 6, and suggests that they may have been preceeded by such a stage.

After the formation of these numerous nuclear vesicles, the fragmentation of the cytoplasm takes place. Figs. 16 and 17, adjacent sections of a seventy-two hour egg, indicate that the fragmentation may take the form of a protoplasmic budding. The large cell shown in the figure has constricted off two fragments and is forming others. Adjoining sections show five additional fragments, making seven in all. Nuclear material is present in only two of the seven fragments. Of course one cannot say that all such fragments are constricted off from the egg, for there is always the possibility that some of them may have come from the polar bodies. It has been shown by Hartman ('16) in the opossum, and by Newman for the armadillo that normally one of the first activities of the unfertilized egg is the unburdening itself of the deutoplasmic material. This is done without assistance of any spindle or apparent nuclear influence. While it cannot be said that the fragments budded off in the mouse are exclusively of yolk material, the process is essentially a similar one.

Figs. 13, 14 and 15 are later stages in the fragmentation process. They represent what Kingery ('13) calls the "morula" stage. Figs. 14 and 15 represent two adjoining sections of the same "morula," which consists of about twenty-one cytoplasmic fragments.

We have seen how the second maturation spindle breaks down and forms either a single resting nucleus or numerous nuclei, and have traced the latter to the "morula" stage. When, on the breaking down of the spindle the egg is able to form a single or only a few resting nuclei, it would seem to indicate exceptional

vitality and a close approximation of the normal condition. In such an egg the process of atresia would naturally be somewhat delayed. The egg with but one or two nuclei does not fragment immediately, and it is possible that such eggs may pass from the body, as suggested by Hartman ('16) for the unfertilized egg of the opossum. This possibility is well illustrated in Fig. 18, which shows an unfertilized egg one hundred and five hours after finding the litter. Only two nuclei are present. The mitochondrial bodies (*b*) can be distinguished from the nuclei by their peculiar crystalline appearance and elliptical shape. Although these bodies are found in most of the fragmented segments to be described later, they are smaller and stain less intensely in hæmatoxylin. They are surrounded by a light area which gives them the appearance of having shrunken from the cytoplasm. In Fig. 19 the mitochondrial bodies are seen to be smaller but more numerous. In the two upper fragments they did not cut cleanly but were pushed aside by the knife. This would seem to indicate that they were much more compact and dense than the surrounding cytoplasm.

Hartman ('16) finds a similar structure in the egg of the opossum, and describes it as a homogeneous, non-granular body staining pink or lavender in iron hæmatoxylin, and bordered by a light-colored band.

The multinucleated eggs soon break up into a number of fragments, often forming two or more parts very nearly of the same size and often appearing like normal cleavage stages, were it not for the presence of extra nuclei. Figs. 12, 13 and 14 show different stages in this process.

The ultimate fate of the fragmented egg will now be considered. Very early in the degenerative process, phagocytic cells are found inside the zona pellucida, between the cytoplasm and the zona. It is impossible to mistake these cells for fragments of either the egg or polar bodies for they appear almost like transparent vesicles with the deep staining nuclear material either scattered as granules or irregularly clumped in a way which immediately suggests their origin as polymorphonuclear leucocytes. Kingery described similar cells but thought them of follicular origin. The number of these phagocytic cells inside the zona varies,

eight or ten being the largest number found. These cells probably aid in the disintegration of the periphery of the cell. This process may begin some time before fragmentation sets in, as is evidenced by a study of Fig. 9. Here the space at one end of the egg filled with dark granules indicates that the cytolysis of the cytoplasm has begun.

The zona persists in nearly all cases as far as the eggs have been followed. Fig. 6 shows one of the exceptions, for here the zona is gone and many dark staining fragments which may have come either from the polar body (which is now very small) or from the broken down zona, are seen surrounding the egg. Several phagocytic cells, one of which is figured, are to be seen among the fragments. Although the zona is present in almost all cases, a careful examination usually brings to light several breaks, and it is probably through these that the phagocytic cells are able to reach the egg.

Figs. 19 and 20 show the last stages in the disintegration and absorbtion of the fragments by the phagocytes. Some of the fragments appear as mere shells with only occasional granules, while the outer edge of others shows a honeycombed condition due to excessive vacuolization.

It is found that the unfertilized egg completes its passage through the Fallopian tube and enters the uterus about the end of the third day or almost at exactly the same time as given by Sobotta for the normal segmenting egg.

Smith ('17), basing ovulation as occurring twenty hours after parturition, finds that unfertilized eggs of the mouse enter the uterus about 76 hours after ovulation and are found in the last fold of the uterus at 88 hours.

The final dissolution of the fragmented egg evidently is completed early on the fourth day, since out of eleven mice older than four days, all showing corpora lutea in the ovaries, indicating that ovulation had taken place, in only three could eggs be found. A single egg was found in the uterus of a mouse one hundred and five hours after parturition (Fig. 18).

In not a single case has anything approaching a cleavage spindle as described in atresic ova by Spuler, Loeb or Newman, been observed, but it is not to be interpreted that they may

not occur, because cleavage spindles are so short lived that the study of a much greater number of eggs would be necessary before coming to any such conclusion.

Summary.

The cytological changes which the unfertilized egg of the mouse undergoes in the Fallopian tube and uterus are closely similar to those which occur in the eggs of atresic follicles in the ovary.

The processes are considered degenerative and have only a superficial resemblance to parthenogenetic development.

The breaking down of the second maturation spindle of the unfertilized egg usually results in the formation of several or many nuclei. Rarely a single nucleus is formed, in which case the egg does not fragment rapidly and may pass from the uterus before the degenerative processes are complete.

The eggs with many nuclei divide into numerous cells, of which some are provided with degenerating nuclei. These are further disintegrated and absorbed by phagocytic cells, which make their way into the egg probably through breaks in the zona. The phagocytes from their appearance are polymorphonuclear leucocytes, and they evidently act on the cytoplasm causing a vacuolization of its outer portions and a later crumbling of its periphery; the end being a complete disintegration of the egg and its absorption by the phagocytes.

Literature.

Athias, M.

'09 Les phénomènes de division de l'ovule dans les follicles de Graaf en voie d'atrésie chez le Lerot (Eliomys quercinus L.). Anat. Anz., Bd. XXXIV. No. 1.

Bonnet, R.

'00 Giebt es Wirbeltieren-Parthenogenesis? Ergebn. der Anat. u. Entwickl., Bd. 9.

Heape, W.

'05 Ovulation and Degeneration of the Ova in the Rabbit. Proc. R. Soc. London, Vol. 76b.

Hartman, C. G.

'16 Studies in the Development of the Opossum Didelphys virginiana. Journ. Morph., Vol. 27, No. 1.

Henneguy, L. F.

'94 Recherches sur les follicules de Graaf chez les mammifères et quelques autres Vertébrés. Journ. d. anat. et d. Physiol., Ann. XXX., No. 1.

Huber, G. C.

'15 The Development of the Albino Rat, Mus norvegicus albinus. I., From the pronuclear stage to the stage of mesoderm anlage; end of the first to the end of the ninth day. II., Abnormal ova; end of the first to the end of the ninth day. Journ. of Morphology, Vol. 26.

Kingery, H. M.

'13 So-called Parthenogenesis in the White Mouse. BIOL. BULL., Vol. 27.

Kirkham, W. B.

'07 The Maturation of the Mouse Egg. BIOL. BULL., Vol. 12, No. 4.

'07a Maturation of the Egg of the White Mouse. Trans. Conn. Acad. Arts and Sci., Vol. 13.

Loeb, L.

'01 On the Progressive Changes in the Ova in Mammalian Ovaries. Journ. of Medic. Research, Vol. 6.

'05 Ueber hypertrophische Vorgänge bei der Follikelatresie, nebst Bemerkungen über die Oöcyten in den Markssträngen und über Teilungserscheinungen am Ei im Ovarium des Meerschweinchens. Arch. f. mikr. Anat., Bd. 65.

'11 The Parthenogenetic Development of Ova in the Mammalian Ovary and the Origin of Ovarian Teratoma and Chorio-epitheliomata. Journ. Amer. Medic. Assoc., Vol. LVI.

'12 Ueber chorionepitheliomartige Gebilde im Ovarium des Meerschweinchens und über ihre wahrescheinliche Entstehung aus parthenogenetisch sich entwickelnden Eiern. Zeitsch. f. Krebsforschung., Bd. 11, No. 2.

Long and Mark.

'11 The Maturation of the Egg of the Mouse. Carnegie Institute Publication, No. 142.

Newman, H. H.

'13 Parthenogenetic Cleavage of the Armadillo ovum. BIOL. BULL., Vol. 25.

Rubaschkin, W.

'06 Ueber die Veränderungen der Eier in den zugrunde gehenden Graafschen Follikeln. Anat. Hefte, H. 97.

Smith, H. P.

'17 The Ovarian Cycle in Mice. (Abstract.) Anat. Record, Vol. 11.

Sobotta, J.

'95 Die Befruchtung und Furchung des Eies der Maus. Arch. f. Mikr. Anat., Bd. 45.

Spuler, A.

'00 Ueber die Teilungserscheinungen der Eizellen in degenerierenden Follikeln des Säugerovariums. Anat. Hefte, H. 50.

Van der Stricht, O.

'01 L'atrésie ovulaire et l'atrésie folliculaire du Follicule de de Graaf, dans l'ovaire de Chauve-Sauris. Verhandl. d. Anat. Gesellsch., 15 Versamml., Bonn.

EXPLANATION OF PLATES.

All the drawings are from sections of unfertilized eggs in the Fallopian tube or uterus. They were made with the aid of a camera lucida, and with the exception of Fig. 5 are reproduced at a magnification of 900 diameters.

Abbreviations.

a, zona. *b*, mitochondrial body. *c*, phagocytes. *d*, vacuole. *e*, degenerating protoplasm.

Plate I.

Fig. 1. (48 hours.) Egg with the second maturation spindle. Polar body shows chromosomes arranged on the spindle fibers. Eggs of this type, showing no changes are found in the tube up to forty-eight hours after parturition.

Fig. 2. (56 hours.) In this egg the spindle is present but the chromosomes seem to have clumped together more than in Fig. 1. The spindle of the polar body has disappeared and in its place are seen two resting nuclei.

Fig. 3. Polar body (60 hours) from same mouse as Fig. 5 showing many resting nuclei formed from the breaking down of the polar spindle or by the chromatin fragments often observed in the polar body.

Fig. 4. (48 hours.) Showing the fragmentation of the polar body. Here the first polar body has divided once and one of the parts has redivided. The spindle cut somewhat obliquely is one of few showing good achromatic fibers. These fibers seem to end in a centrosome-like body.

Fig. 5. (60 hours.) Chromosomes of the second maturation spindle breaking down and forming resting nuclei. Vesicular lobes are seen extending out from the main vesicle. Quite a number of the chromosomes have not yet entered into the process and are seen in the lobes of the forming nuclei. This mouse had a tumor in one of the uteri which prevented fertilization, while on the other side, eggs in the tube had reached the four cell stage. (× 1,800.)

Fig. 6. (78 hours.) A later stage of vesicle formation from the maturation spindle. Chromatin all in the vesicles, no chromosomes as such. This was one of the few eggs in which the zona was missing. Many dark staining granules surrounded the egg and in among them several phagocytes. One phagocyte shown at left of polar body, which is much smaller than normal, suggesting that the phagocytes have been acting upon it and probably upon the zona also.

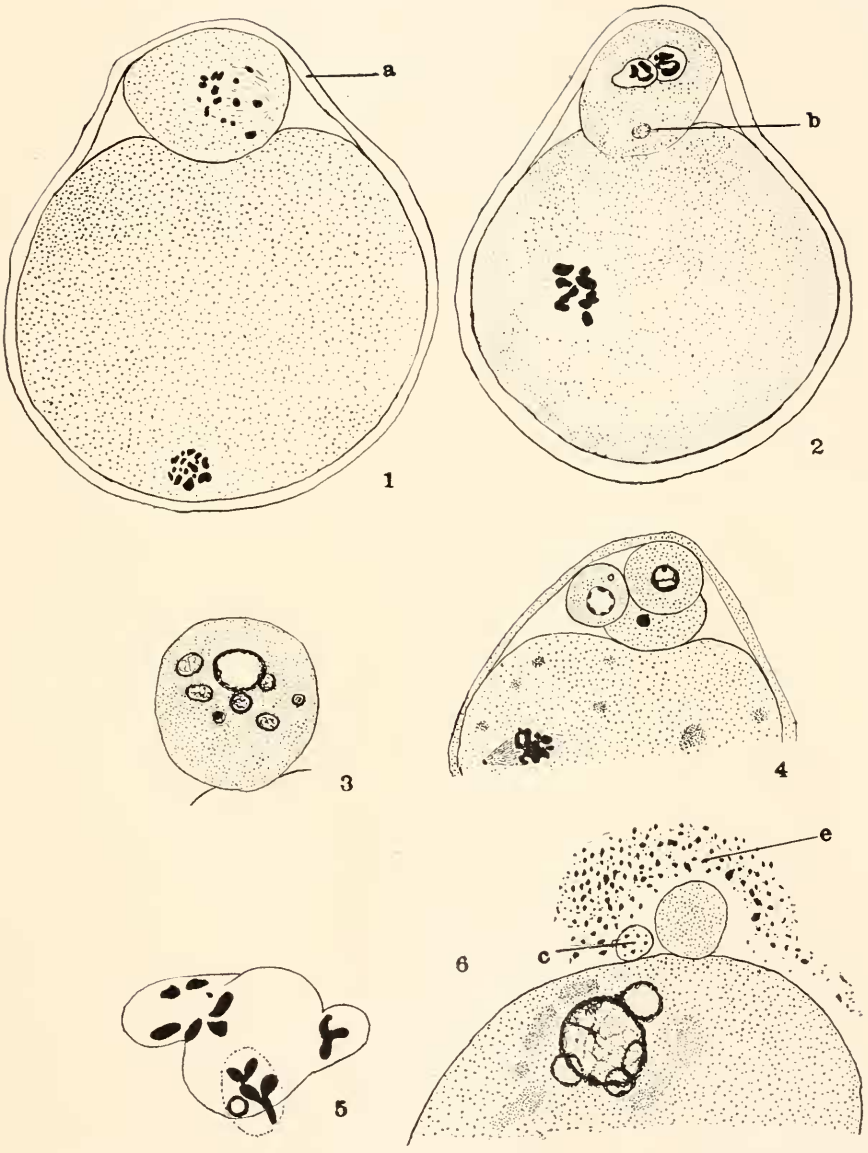

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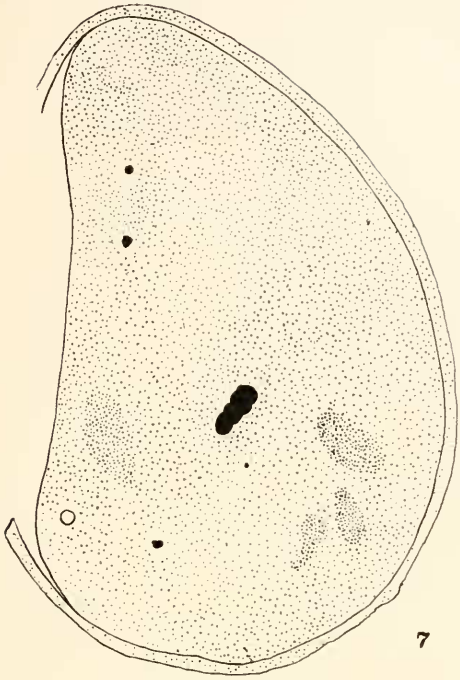
Plate II.

Fig. 7. (72 hours.) Spindle fibers missing, chromosomes of spindle hugged closely together. Several parts separated and out in the cytoplasm.

Fig. 8. (72 hours.) A later stage of the above. Chromosomes scattered in the cytoplasm. One showing the beginning of vesicle formation. Several phagocytes are present inside the zona. Cytoplasm near open end shows beginning of disintegration.

Fig. 9. (72 hours.) Completion of vesicle formation begun in Fig. 8. The chromatic material clumped to one side of the vesicle. Quite a few small particles of disintegrated cytoplasm may be seen inside the zona indicating the activity of the phagocytes.

Fig. 10. (72 hours.) Reconstruction showing ring-like formation of nuclear material. Possibly a succeeding stage to Fig. 6. A phagocyte cell is noticed just outside the zona, which is very faintly stained.



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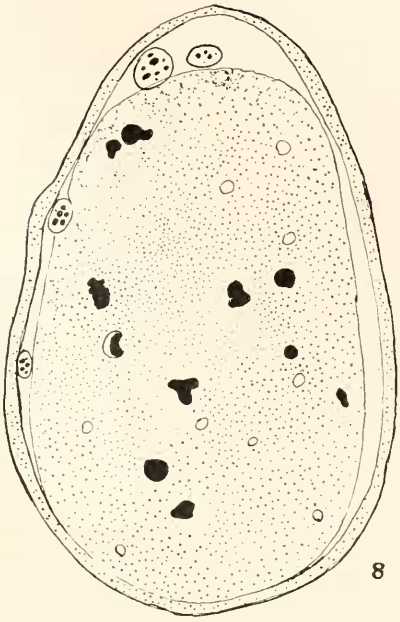

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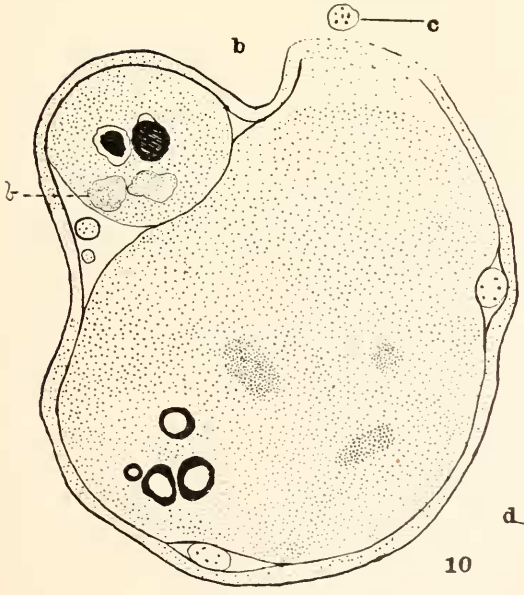

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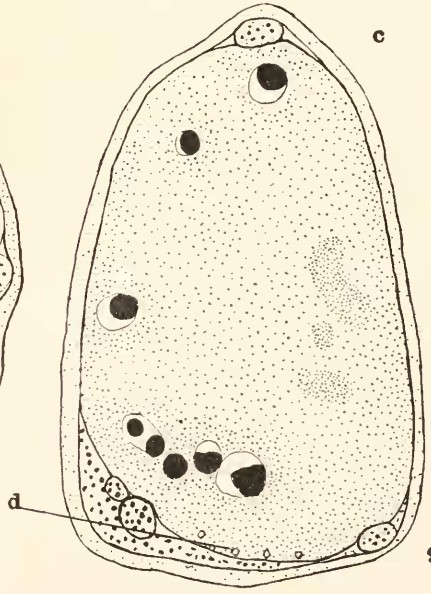

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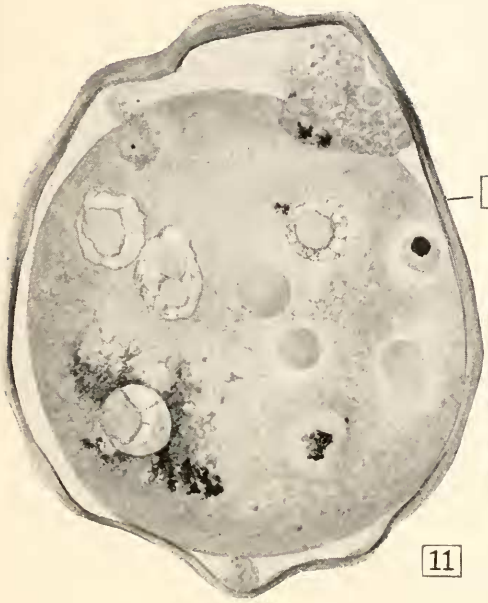
PLATE III.

FIG. 11. (72 hours.) Egg showing four resting nuclei, and a number of mitochondrial bodies. In addition a divided polar body and two or more smaller structures which are probably phagocytic cells.

FIG. 12. (72 hours.) Fragmented egg looking very much like a normal two-cell stage, except that each blastomere has two or three nuclei. The nuclei contain nucleoli and appear perfectly normal.

FIG. 13. (81 hours.) A later stage of fragmentation. A large resting nucleus is seen in one of the larger segments, while other fragments have none. In two of the cells the mitochondrial body is quite conspicuous.

FIG. 14. Egg of uncertain age, consisting of twenty-one fragments. Numerous phagocytic cells are seen in and about the fragments. Zona entirely missing.



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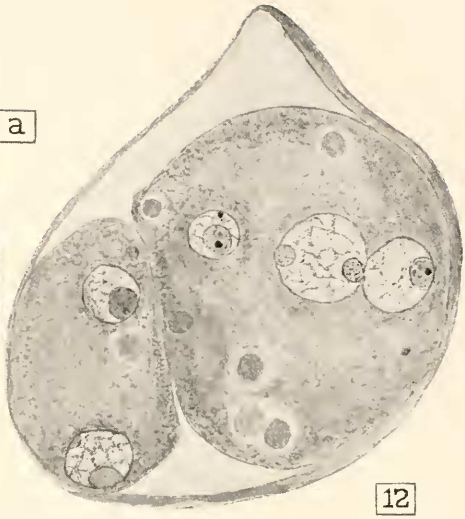

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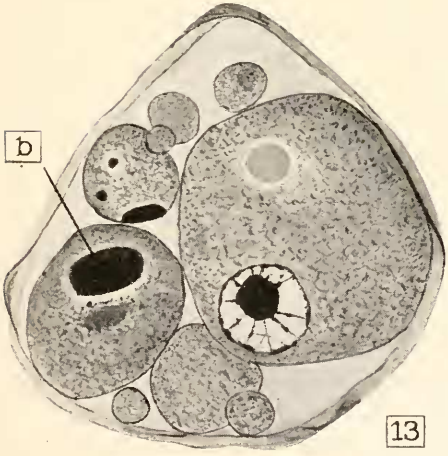

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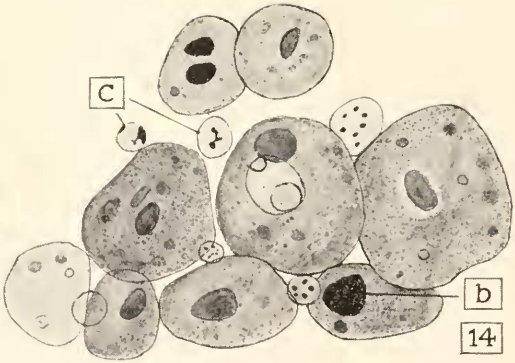

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Plate IV.

Fig. 15. Drawing of adjacent section to that shown in Fig. 14.

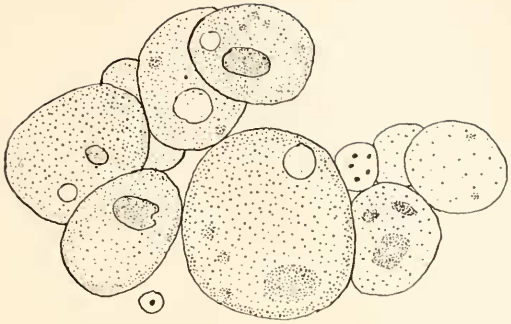
Fig. 16. (72 hours.) Egg showing the formation of protoplasmic fragments independent of any spindle. These are later pinched off and form small segments inside the zona.

Fig. 17. Drawing of adjacent section to that shown in Fig. 16. A study of all the sections of this egg shows that seven fragments have been pinched off, and that of these only two appear to contain nuclear material.

Fig. 18. (105 hours.) Egg is without zona, contains two nuclei and two immensely large refractive mitochondrial bodies. The polar body still remains attached to the egg.

Fig. 19. An 81-hour egg showing phagocytic effect. In two upper cells the mitochondrial bodies have been pulled out of the cytoplasm towards the left by the knife, leaving clear spaces where they originally were, indicating the solid character of these structures. Many vacuoles are seen around the border of the fragments. Between the cells dark stained particles of degenerated cytoplasm is being absorbed by the phagocytes.

Fig. 20. (81 hours.) A later stage than Fig. 19. In one of the fragments hardly any content is to be seen except a few scattered granules.



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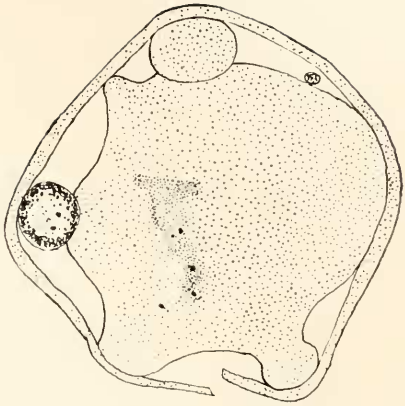

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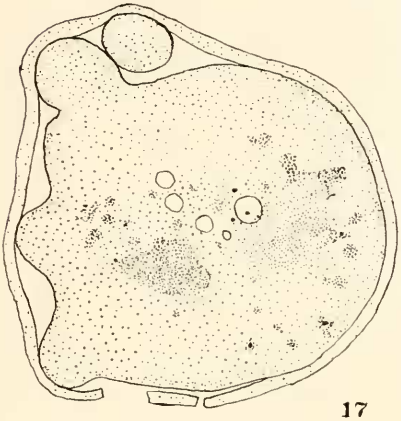

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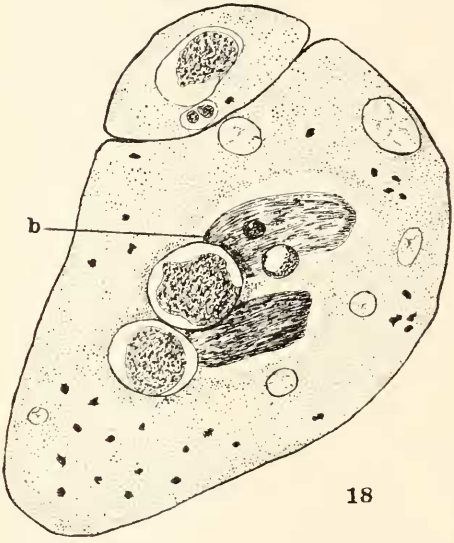

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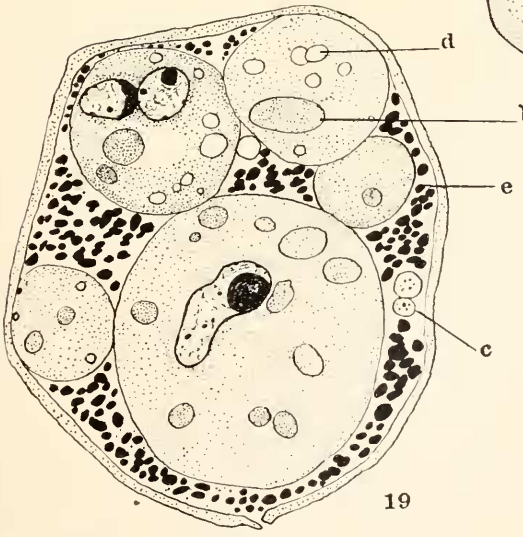

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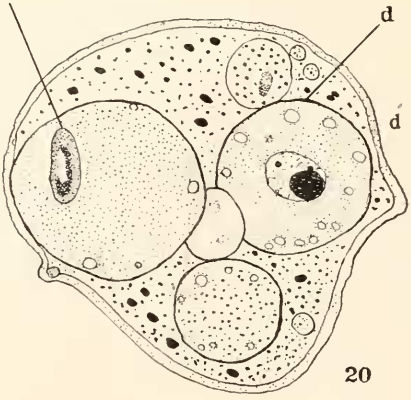


20

OBSERVATIONS ON THE STRUCTURE OF DOUBLE MONSTERS IN THE EARTHWORM.[1]

ROXIE A. WEBER.

I. Introduction.

The work which has thus far been done on double monsters in the terricolous group of annelids is quite limited, since in only a few species, *Lumbricus trapezoides*, *Lumbricus terrestris*, *Allolobophora fœtida* and *Allolobophora trapezoides*, has this condition been observed. The first of these forms is described by Kleinenberg, the remaining three by Vedjovsky. These two investigators agree neither as to the method of union nor as to the cause of this phenomenon. It is not the object of this paper to give the factors influencing such monster formations, but merely to describe the manner in which the members are joined.

Material and Technique.—The material studied consists of twelve double embryos, the shortest member of which is at least 60 segments in length, the longest 125, one monster in which one individual consists of 16 the other 17 segments, 19 double gastrulæ and two embryos showing a bud on one side of the blastopore, and one case of an egg consisting of two distinct hemispheres connected by a band of large cells. The entire supply was obtained from Professor Patterson, of this University.

The fixing fluids employed were Meves, Gilson, and Bouin, all of which gave only fairly good results. Ehrlich's hæmatoxylin was used as a stain.

The species studied is *Helodrilus caliginosus trapezoides*, according to Professor F. Smith's identification.

Review of the Literature.—Before describing the individuals studied, it will perhaps be well to give a brief review of the results of the two authors above mentioned.

Kleinenberg found that the number of eggs in the capsules of *L. trapezoides* varies from three to eight of which usually one,

[1] Contributions from the Zoölogical Laboratory of The University of Texas, No. 139.

though sometimes two or three, produces an embryo. The remaining ones, not becoming fertilized, disintegrate completely.

By a series of somewhat irregular cleavages the egg develops into a germinal bladder, one layer in thickness (Pl. IX., Fig. 2, Kleinenberg). Thereupon a two-layered condition begins to appear, one side of the cell mass becoming differentiated in advance of the other. At this pole all the cells divide rapidly except two, which become pushed in and covered over by the small blastomeres. From these two cells (mesoblasts) are derived the first rudiments of entoderm and mesoderm (Pl. IX., Fig. 4).

While this elongation is taking place a transverse furrow appears midway between the two ends extending almost entirely around, leaving the two hemispheres connected by only a few enlarged ectodermal cells.

When this stage has been reached the cells of the other pole begin to undergo the same changes and finally we have formed from each half an embryo joined in varying degrees to the other by a band of ectodermal cells. The separation is eventually effected by a series of rotations which usually result in the breaking of the uniting cord. When for one reason or another this is not accomplished we have true monstrosities in all degrees of coalescence.

According to Kleinenberg the union never extends to the internal organs but is confined to the external epithelium of the body wall.

The monsters formed in this manner are of the same or nearly the same size. There is however still another type of abnormality (Pl. IX., Fig. 10), *e. g.*, those showing bud formation. Kleinenberg explains their production as being due to a very unequal development of the two halves of the above mentioned cell mass.

In all his work he has found only a few cases in which two individuals did not emerge from one capsule and in those few exceptions rudiments of a second were usually found. For this reason he concludes that each egg produces two individuals normally.

Vedjovsky on the other hand holds this condition to be ab-

normal. He explains the formation of double embryos as being due to what he terms *Doppelfurchung*. The egg elongates in the direction of the animal pole and subsequently divides into two blastomeres either of equal or unequal size. They are designated as A and a. From each of these cells there is produced a quartet, one lying upon the other giving the appearance of micro- and macromeres in the cleavage of *Crepidula* or the eight cell stage of *Synapta*, depending on the proportionate sizes of A and a. Each of the quartets finally gives rise to an individual which remains attached to its companion. If the a cell is smaller than A, there is produced one well developed individual and a bud.

This author has found examples of monster formation in *Lumbricus terrestris*, *Allolobophora fœtida* and in *Allolobophora trapezoides*. In the first group he found two cases in both of which the two individuals were joined on the dorsal side through one segment. Only one monster was found among several hundred embryos of the second group. In the third group a large number was found which the author describes under three divisions.

A. Those monsters in which the individuals are united on the ventral side along the entire length of the body (Pl. 19, Fig. 14).

B. The cases in which the members are joined on the dorsal side (Plate 21, Fig. 7).

C. The double monsters in which the individuals are fused end to end. (*a*) Those in which both members are of similar length (Pl. 21, Fig. 9). (*b*) Those cases in which one member is rudimentary (Pl. 19, Fig. 12).

II. Observations.

Even though these two workers differ so markedly in their conclusions it will be noted from the following that the observations made by the writer are similar in many respects to those made by both of these men.

In this study of the monsters of *Helodrilus caliginosus trapezoides* it has been found that the greatest variety exists in the structure of the nervous system. There is in every case a complete union of the five layers of the body wall, and the

digestive tract is either fused or not fused, but the nervous system is modified in a variety of ways. The manner and extent of union of these individuals and their organ systems are best described in the following groups.

1. Those double monsters in which the union is dorsal. This group may again be subdivided into—

(*a*) Those in which the union extends to the alimentary tracts. Of these there are two examples, Nos. 95 and 171, Plate I. From Figs. 1 and 2, Plate I., it can be seen that the fusion in No. 171 is through one segment only, while in No. 95 it extends through five. A description of sections through this monster will serve to show the general relationship of the organ systems in the members of this group. Fig. 3, Plate I., is a section through the anterior portion showing the condition of the nervous system. It will be seen that there is almost a continuous band of nerve tissue extending around the pharynx. A study of the neighboring sections shows that this band is really complete. It will also show that the portion marked (*b*) in the figure enlarges into a bilobed brain and that a similar structure exists on the opposite side of the pharynx. At the same time there is also to be found at each end of the greatly elongated pharynx another set of ganglia. It would be impossible here to determine which are brain and which are ventral cord ganglia were it not for the presence of setæ (*s*). This at once leads to the conclusion that the ganglia at the end of the pharynx must be those of the ventral cord. This inference is further proven by a study of sections through a more posterior region, as shown in Fig. 4, Plate I. Here are no longer to be found the ganglia on either side of the pharynx but only those at the opposite ends. This section too shows the setæ in normal relationship with the ventral cord and the nephridæ in their natural position. They are shown only on one side but those of the other half can be seen in the next section.

It will be noted that the single pharynx is greatly elongated from end to end of the monster. Throughout the fused part it remains as one cavity dividing into two parts only when the point of separation of the two members is reached, one passing into each individual. Surrounding the pharyngeal cavity there

is a great mass of muscular tissue attached by thin strands to the body wall. This condition is exactly similar to that found in single individuals.

The sub-intestinal blood vessels are clearly visible (*v.b.v.*) in their normal position between the alimentary tract and the nerve cord. On each side of the muscular pharynx there lies a more or less regular vessel (*D.B.V.*) which when traced to the point of separation of the two members will be found to approach the center and then one passes into one individual, the other into the other, the vessel on the left side passing into the upper half, the one on the right into the lower half.

Though the other monster is formed by a coalescence of the two members through one segment only, that union is complete in every respect from body wall to pharynx. Fig. 5 is a longitudinal section through No. 171 showing a portion of one of the brain ganglia together with a small part of the nerve cord of each of the individuals. A study of the successive sections will show that the pharynx arises from a flattened portion which is lined with the same kind of epithelial cells as the rest of the digestive tract and which lies between the two members as indicated in Fig. 2, Plate I. (*m*). The ventral cords are found to be connected to the cerebral ganglia, of which there are two sets, one on each side of the mouth opening, by means of commissures as in No. 95.

The setæ, not shown in this figure, and the nephridæ lie along the same side of the body as the nerve cord.

(*b*) Those in which the alimentary tracts have not become united. Nos. 91, 92, 142 and 173 shown in Plate II as Figs. 6, 7, 8 and 9 illustrate this group. It will be noted that though only one definite pair of segments appear to be fused in the first three cases, there is in each instance an irregular mass of tissue between the separated edges of the fused pair of segments. Whether these masses are modified segments has not been determined.

In all four instances the nerve cords are formed on the side opposite the line of fusion. These cords are joined to the bilobed brains in a manner very similar to that found in No. 95 (Fig. 3). It is to be noted that the union of these individuals

is the same in kind and extent as in the group already described except for the digestive tract. In every one of the four there is distinguishable a separate alimentary tract for each member beginning with the mouths. These structures lie very near the nerve cord and are bounded on the inner side by a mass of muscles similar to those which usually lie on either side of the elongated fused pharynx.

The fixation of the blood vessels was such that they could not be studied.

The members of these groups to which belong half the specimens studied, have undoubtedly been fused along the dorsal side. The relative positions of setæ, nephridæ, and nerve cord are positive proof thereof. Vedjovsky gives a number of figures, very similar to Figs. 3 and 4, in his paper but he explains the union as being along the ventral side. It is impossible to disprove this conclusion for the two members were joined along their entire lengths. In the cases described in this paper, the union was never through more than five segments If, then, the coalescence had been other than dorsal, it is very probable that the nerve cord together with the setæ and excretory organs would have swung around to their natural position in the separated portions of the two individuals. This, however, is not the case. Therefore, the conclusion must be drawn that at least in these instances the union is no other than dorsal.

It is very probable that the one case described by Vedjovsky is also one of dorsal instead of ventral union, and that the cerebral ganglia are composed of half from each member instead of this being the case for the ventral cords, as that author supposes. If those individuals in which the digestive tract was separate had not been found it would not be possible to make this statement for it might be argued that each side of the pharynx was formed from the alimentary tract of one member. But since those cases have been found it can be easily seen that a joining of these structures would have resulted in forming a greatly elongated organ similar to that shown in Vedjovsky's figures. Furthermore, if the union in these forms had been ventral, the pharynges would certainly not have been found in the positions in which they lie, but would have been much nearer together in the central portion and side by side instead of end to end.

2. Those cases in which the union is latero-dorsal. Thus far only one example has been found of this group, individual No. 70 shown in Fig. 10, Plate II. Figs. 11 and 12, Plate II., show sections through the joined region which in this case extends through three segments. The first is very much more anterior than the second as is indicated by the presence of the cerebral ganglia (*bg*) of which there is in this monster only one set. The commissures extending from it can be seen in the same figure in which is also present the beginning of one of the nerve cords (*nc*). A study of the next few sections shows the connection of the brain to the two cords and also a connecting band between these two structures. In this manner the single greatly elongated alimentary tract, which in this case is the result of a union, is completely surrounded by nerve tissue. Fig. 12 gives a section through a more posterior region showing the relative positions of the ventral cords.

In no part of the joined portion can there be found less than eight pairs of setæ indicating that the union does not extend beyond the sides of the individuals.

The nephridæ also are in their normal number and relationship to the ventral cord.

3. This is the group in which the union is end to end and in which the cerebral ganglia are to be found on the opposite side of the digestive tract from the ventral cord. It can be seen from an inspection of Figs. 13, 14 and 15, external drawings of Nos. 90, 93 and 2, that the extent of union is limited to an unusually small area. Sections show that the union is across the dorsal side, for the ventral cords lie on the sides opposite that area, while the cerebral ganglia lie on the same side.

The digestive tracts have a common origin in each of the three monsters arising from a single mouth opening from which the pharynx passes into each individual. There is no noticeable difference in the structure of any of the fused organs of these forms as compared with those of No. 95 except in the nervous system.

A study of No. 90 will show a condition of that system somewhat different from any so far described. The ventral cord of each individual lies on the side opposite the union while the

brains lie on the same side and are connected to the cords by means of commissures, a condition very similar to that found in normal single individuals. The brains however in this instance are joined to each other. In one of the individuals the commissural connection between brain and cord can be seen beyond the point of separation, but in the other member the two ganglia of the brain seem to be separated and joined separately to the cerebral ganglia of the first member.

In No. 93, shown in Fig. 14, there is present only one set of cerebral ganglia greatly elongated. It lies on that side of the pharynx opposite the cords and is joined to that structure in one member by two commissures, to that in the other by only one.

A study of sections through No. 2 reveals a condition similar to that in No. 90. There are two distinct sets of cerebral ganglia, one joined to each nerve cord as in normal individuals and a connection between the two brains much as between the two cords in No. 70.

4. That group of double monsters in which the union between the two individuals has been side to side with both mouth openings on the same side, illustrated by Nos. 67 and 1.

In No. 1 the union is through one segment only as indicated in Fig. 16, Plate III. There is a single mouth opening lined with the columnar epithelial cells, from which the digestive tract of each individual passes inward. There can be seen on one side of the pharynx in a transverse section a single set of cerebral ganglia which is joined to the ventral cords by commissural strands extending over the pharynx. There are no such structures to be found on the under side of the alimentary tract. On the opposite side of the section there can be traced a connection between the cords themselves. Fig. 17, Plate III., is a section through the united portion of No. 1 showing the single pharynx, cerebral and ventral ganglia and a portion of the strand of nerve tissue connecting the two cords.

A study of sections through No. 67, shown in Fig. 18, Plate III., will show that the two mouth openings have been joined into one, and that the ventral cords lie on one side of the common pharynx and the cerebral ganglia on the other. There is only one set of brain ganglia to be found in this monster, but,

unlike all other cases, there are three distinct commissural strands joining it to the nerve cords, one extending over the top of the pharynx, the other two below the point of union of the two digestive tracts forming a triangle with each other. Fig. 19, Plate III., is a cross section of these last mentioned strands and their connection to the two cords. As in No. 70 there is in this monster also a limited connection between the two cords.

5. Cases in which the two individuals are extremely unequal in size, *e. g.*, bud formation. Thus far there have been found two such cases, a section of one being shown in Fig. 20, Plate III.

III. Discussion.

It will be remembered that reference was made to the difference in view concerning the origin of double monsters in an earlier part of this paper. Vedjovsky maintains that the formation of two embryos from one egg is abnormal, whereas Kleinenberg maintains the opposite. A difference of this kind may be explained on the basis of the difference in the forms worked on by these two investigators. This paper is a study of a form very similar, in its mode of development, to that described by Kleinenberg. It has been found that it is quite common, not only for monsters to appear in this group, but also for two individuals to emerge from one capsule. Out of the 184 cocoons opened 57 contained only one individual, 101 two, and 25 eggs in various cleavage stages and 1 four embryos. Thirty-five of the 101 cases were in the form of monsters. Six cases have been noted in which two or more eggs were present but in four of these only one was undergoing development while the others were in various stages of degeneration. One egg was found which had just reached a stage in development sufficiently far advanced to show the division into two hemispheres and the connecting band of larger cells very similar to Kleinenberg's Fig. 6. Three others were found which had begun to elongate in a manner very similar to the above.

It would certainly be safe to conclude that it is a common occurrence for one egg of this species to give rise to two individuals. If this were not the case it would seem rather unusual to find so large a number of cases in which two and only two

individuals emerge from one capsule when the number of eggs found in one capsule varies ordinarily from three to eight. The elongation of those eggs sufficiently far advanced in development to show this phenomenon is still further indication of this same thing.

Kleinenberg attributes the cause of this double embryo formation to the entrance of two sperm into a single egg setting up two points of activity. In the light of more modern discoveries this explanation no longer holds good. Vedjovsky suggests the possibility of temperature and moisture changes, and exposure to air bearing their influence on the egg and causing its abnormal development. Until further studies are made along this line it will be impossible to state any definite causes of monster formation.

BIBLIOGRAPHY.

Kleinenberg, N.

'78 Sullo Sviluppo del Lumbricus trapezoides.

Vejdovsky, Fr.

'88–'92 Ueber Zwillingsbildungen der Lumbriciden. Entwicklungsgeschichtliche Untersuchung.

Explanation of Plate I.

Helodrilus caliginosus trapezoides.

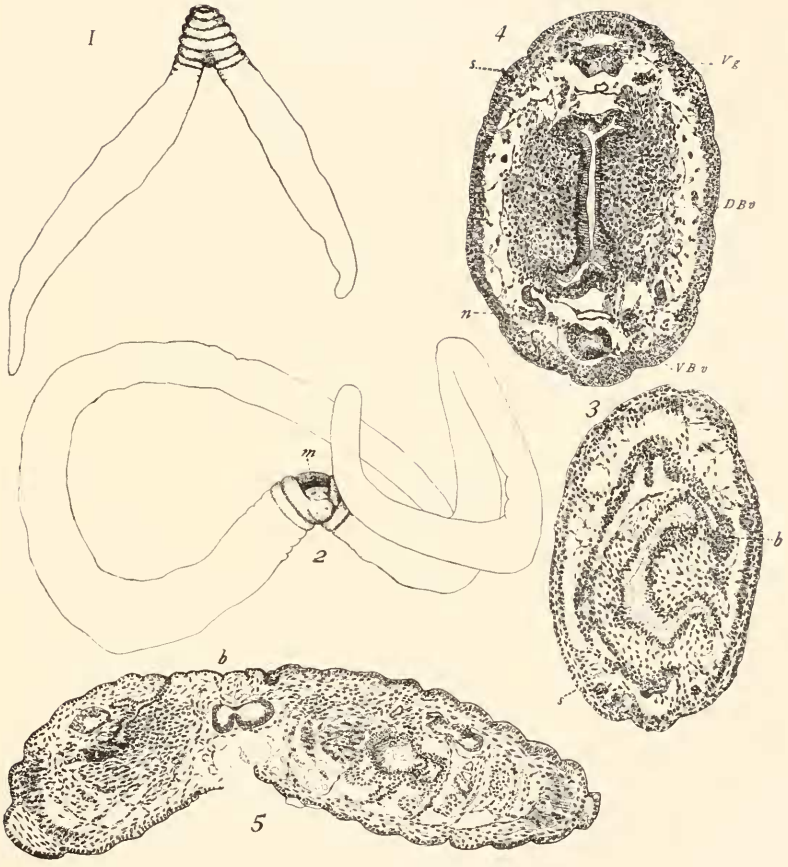
1. No. 95, showing union through five segments.

2. No. 171, showing union through one segment.

3. Section through the anterior portion of No. 95, showing the pharynx fused and the extension of the nerve tissue almost entirely around the pharynx. *b*, cerebral ganglia; *s*, setæ.

4. A section through a more posterior region of the same monster. *Vg*, ventral ganglia; *DBv*, dorsal blood vessel; *VBv*, ventral blood vessel; *n*, nephridium; *s*, seta.

5. A longitudinal section through No. 171, showing a portion of the cerebral ganglia *b*, and also parts of the ventral cords.



ROXIE A. WEBER.

Explanation of Plate II.

Helodrilus caliginosus trapezoides.

6. No. 91.
7. No. 92.
8. No. 172.

} Fused through one segment only with irregular masses between the separate edges of the united segment.

9. No. 173, united through four segments.

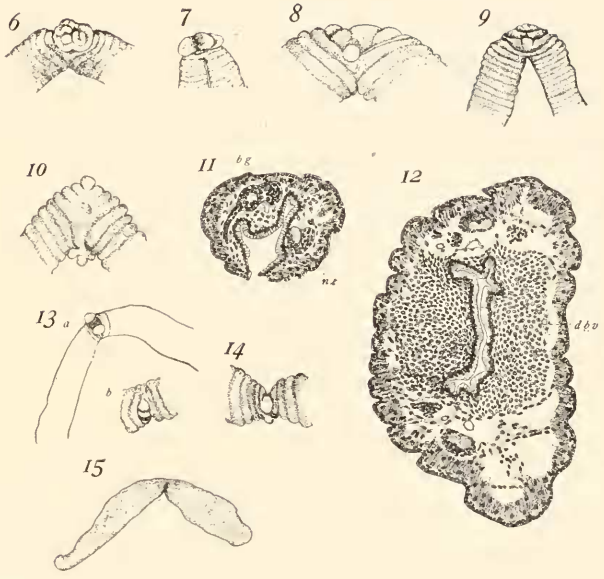
10. No. 70, united through four segments.

11. A section through the anterior portion of No. 70, showing a single set of cerebral ganglia (*bg*) and one of the ventral ganglia (*nc*).

12. A more posterior section of the same monster showing two ventral ganglia.

13*a* and *b*. No. 90.
14. No. 93.
15. No. 2.

} All show very limited connection.



ROXIE A. WEBER.

Explanation of Plate III.

Helodrilus caliginosus trapezoides.

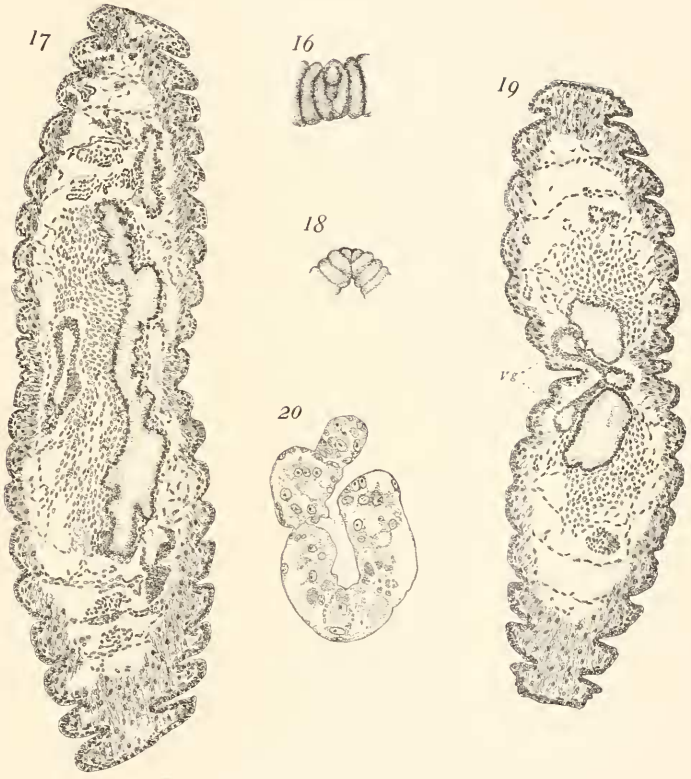
16. No. 1 viewed from the side of the mouth opening.

17. A section through No. 1 horizontal to the mouth opening, showing the cerebral ganglia on one side of the pharynx and the cord tissue on the opposite side.

18. No. 67, united through two segments.

19. A transverse section through the posterior part of the united portion showing the cerebral ganglia joined to the ventral ganglia (*vg*) by commissures.

20. A section through a gastrula stage showing bud formation.



ROXIE A. WEBER.

IODINE AS A PARTHENOGENETIC AGENT.

ALVALYN E. WOODWARD AND FLORENCE S. HAGUE.

In the course of some experiments with *Arbacia* eggs at Woods Hole last summer, it was noted that, if a little iodine was added to the eggs in sea-water, regular fertilization membranes appeared on a large number. This led to an attempt to find the optimum conditions for the use of this reagent in parthenogenesis.

The stock solution used was made by dissolving iodine crystals in sea-water to the point of saturation. This stock solution was diluted with sea-water as shown in the tables.

Some of the females were stimulated to shed their eggs into watch glasses. In other cases, the ovaries were removed and shaken in sea-water to loosen them. Whichever method was followed, the eggs were washed and allowed to settle. The supernatant water was poured off, the eggs evenly mixed in fresh sea-water, and 2 c.c. of the mixture put into each of a series of watch glasses. To each of these an equal volume of iodine solution was added, for a given length of time. Some of the eggs were then put into finger-bowls containing about 50 c.c. of sea-water. Others were treated for 20 minutes with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2.5 N NaCl). At the end of that time, the solution was drawn off and the eggs transferred to finger bowls of sea-water. With each lot of eggs, three controls were made, one fertilized with sperm, one uninseminated, and one uninseminated but treated with hypertonic sea-water.

Table I. shows the percentage of membranes and cleavages produced by varying the strength of the solution and the length of exposure. To obtain these averages, over two hundred eggs were counted in each case.

It will be noted that there is great variability in the results with different batches of eggs. This may be explainable from the fact that the experiments were carried on during the last two weeks of August and the first week of September, a period

TABLE I.

PERCENTAGE OF CLEAVAGES AND MEMBRANES OBTAINED BY TREATING ARBACIA EGGS WITH IODINE, OR IODINE FOLLOWED BY HYPERTONIC SEA-WATER.

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Sea-water.	Not Fertilized. In Hypertonic.	
2.5			14	24											69	3	5	Shed
			14	22											64	2	2	Shaken
	2	7	17	16											68	4	2	Shaken
					26	27	16	16							41	1	4	
					16	32	11	39							69	3	11	
									12	13	12	14			85	7	9	
									5	8	4	12			83	7	9	
5					16	12	14	39	6	16	3	3			44	2	2	Shed
					18	21	30	22	17	17	5	15			32	1	2	Shed
					3	15	20	22	20	23	9	10			83	1	4	Shaken
			1	1	21	2	20	29	14	23					82	5	6	Both
							12	32							3	1	1	Shed
							19	20							94	5	6	Shaken
							16	27							94	5	6	Shaken
									8	21					66	3	5	Both
			20	17											69	3	5	Shed
			19	7											64	2	2	Shaken
	1	1	18	9											68	4	2	Shaken
					49	49	11	5							41	1	4	
					23	37	26	43							69	3	11	
									10	13	9	4			85	7	9	
									2	13	5	10			83	7	9	

TABLE I.—*Continued.*

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Seawater.	Not Fertilized. In Hypertonic.	
7·5			5	21	29	20	16	13	1	2					13	0	1	Shed
			35	37	28	28	41	31	8	18					70	4	10	Shaken
							21	47							3	1	1	Shed
							30	22							94	5	6	Shaken
							22	27							94	5	6	Shaken
									9	26					66	3	5	Shed
			15	13											69	3	5	Shed
			11	10											64	2	2	Shaken
	7	4	23	16											68	4	2	Shaken
					34	24	24	27							41	1	4	
					25	36	28	27							69	3	11	
10			13	7	23	20	21	19	11	16					88	1	5	Shed
			0	15	30	26	25	19	8	21					51	0	3	Shed
			3	9	37	25	13	33	7	17					88	1	11	Shaken
			3	15	22	16	14	18	11	6					13	0	1	Shed
			28	23	19	23	11	21	23	32					70	4	10	Shaken
			1	1	1	8	26	31	28	28					82	5	6	Both
							45	56							3	1	1	Shed
							19	24							94	5	6	Shaken
							26	37							94	5	6	Shaken
									16	24					66	3	5	Both
			8	5											69	3	5	Shed
			7	14											64	2	2	Shaken
	3	3	11	17											68	4	2	Shaken

TABLE I.—*Continued.*

Time. Min	Sat. Iodine Solution.	+ Hypertonic.	½ Sat. Iodine Solution.	+ Hypertonic.	¼ Sat. Iodine Solution.	+ Hypertonic.	⅛ Sat. Iodine Solution.	+ Hypertonic.	1/16 Sat. Iodine Solution.	+ Hypertonic.	1/32 Sat. Iodine Solution.	+ Hypertonic.	1/64 Sat. Iodine Solution.	+ Hypertonic.	Controls. Fertilized.	Not Fertilized. In Seawater.	Not Fertilized. In Hypertonic.	Remarks.
10					32	44	18	14							41	1	4	
					26	39	18	44							69	3	11	
									15	12	4	15			85	7	9	
									6	17	4	10			83	7	9	
12.5							8	28							3	1	1	Shed
							13	18							94	5	6	Shaken
							23	24							94	5	6	Shaken
									16	26					66	3	5	Both
			12	6											69	3	5	Shed
			7	19											64	2	2	Shaken
	11	3	8	16											68	4	2	Shaken
					18	20	19	46							69	3	11	
15							15	33							3	1	1	Shed
							16	22							94	5	6	Shaken
							17	41							94	5	6	Shaken
									21	35					66	3	5	Both
					24	42	29	29							69	3	11	
									9	11	10	12			85	7	9	
					21	27	7	13					2	5	92	1	2	Shaken
20									15	17	14	15			85	7	9	
					21	24	9	5					2	8	92	1	2	Shaken
25									15	20	8	16			85	7	9	

near the close of the breeding season, when the eggs vary greatly in their fertilizing power and their sensitiveness to reagents. It is probable that the strength found best for this season will prove too great for the more sensitive eggs in the height of the season—July, for instance.

Table II. is a summary of Table I., giving the average number

TABLE II.

SUMMARY OF TABLE I.

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.	
		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.
2.5	'2	7	15	21	21	30	14	28	9	11	8	13		
5	*1*	*1*	15	9	*22*	*23*	19	27	11	18	6	8		
7.5	*7*	*4*	18	19	29	27	26	28	6	15				
10	*3*	*3*	6	12	24	25	20	26	14	19	4	13		
12.5	*11*	*3*	9	14	*18*	*20*	16	29	*16*	*26*				
15					23	35	17	28	15	23	*10*	*12*	*2*	*5*
20					*21*	*24*	*9*	*5*	*15*	*17*	*14*	*15*	*2*	*8*
25									*15*	*20*	*8*	*16*		

of cleavages plus membranes obtained by each method of treatment. A number in italics is the result of one experiment, rather than the average of several. There is a remarkable uniformity in the percentages obtained by treating the eggs for varying periods of time. For instance, whether exposed for two and one half or fifteen minutes, or for some intermediate period, to one eighth saturated iodine, the eggs gave percentages between 26 and 28. The indication is that the iodine enters the egg immediately and affects it immediately to its full extent. One would expect this result if it acted by combining chemically with something in the egg. While length of exposure has no effect, varying the strength of iodine used causes great variation in the result. This indicates a secondary, non-initiatory or injurious effect due to the excess. The optimum results usually followed treatment of 2 c.c. eggs in sea-water with 2 c.c. of one fourth or one eighth saturated iodine solution. While hypertonic after treatment was nearly always beneficial, in most cases the effect was so slight as to be negligible.

It may be recalled that membrane formation and subsequent

cytolysis were obtained by Loeb,[1] McClendon,[2] and Glaser,[3] in *Arbacia* eggs, by placing them in sea-water diluted with distilled, or in pure distilled water. In the latter case, the eggs became "ghosts" of their former selves, since the pigment dissolved out and increased osmotic pressure caused the egg to absorb water and swell enormously. If an excess of iodine is added to normal eggs, they also become pale, but due to a precipitation of the pigment, which gathers as a small dark mass near one side of the egg. There is seldom indication of swelling or ghost formation.

The membranes obtained by the iodine method were identical with the true fertilization membranes which appear when the sperm enters the egg. They do not resemble the membranes formed by treatment with butyric acid and hypertonic sea-water, which Loeb describes as very difficult to see, excepting by the trained observer. This normal appearance of the membranes was noted by others accustomed to distinguishing types of membrane in *Arbacia*. The significance of the nature of this membrane will be discussed in a later paper

Reference to Table I. brings out a result that was wholly unexpected. It is commonly supposed that eggs shed by the animal are in better condition and more fertile than those taken from the ovary. The results with one female, whose shed eggs produced only 3 per cent. cleavages, while those shaken from the same ovaries gave 94 per cent., were so striking that the averages were computed. It was found that the shed eggs averaged only 43 per cent. as against 80 per cent. cleavages from the eggs shaken from the ovaries. It is planned to test this during the height of the breeding season, so as to discover whether this is a normal occurrence or whether the "shed" eggs are more likely to be "over-ripe" than the others, late in the season.

[1] Loeb, Jacques, "Die chemische Entwicklungserregung, etc.," Julius Springer, Berlin, 1909.

[2] McClendon, J. F., "On Artificial Parthenogenesis of the Sea-urchin Egg," *Science*, 1909, Vol. 30, pp. 454–455.

[3] Glaser, O. C., "On Inducing Development in the Sea-urchin (*Arbacia punctulata*), Together with Considerations on the Initiatory Effect of Fertilization," *Science*, 1913, Vol. 38, pp. 446–450.

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BIOLOGICAL BULLETIN

SELECTION FOR HIGHER AND LOWER FACET NUMBERS IN THE BAR-EYED RACE OF DROSOPHILA AND THE APPEARANCE OF REVERSE MUTATIONS.[1]

H. G. MAY.

CONTENTS.

THE EXPERIMENTS OF ZELENY AND MATTOON.

In 1915 Charles Zeleny and E. W. Mattoon published their results obtained by selecting for higher and lower facet numbers in the bar-eyed race of *Drosophila*. Three lines were selected for higher numbers and three lines for lower numbers. Individual pairs were mated and the selection was carried on for three generations in each direction.

They counted the facets in 250 flies at the beginning of the experiment and found the average number to be 98.04 for males and 65.06 for females. Counts made on 250 more flies from the stock at the end of the experiment gave the same results. From the ratio between numbers from males and females they computed a factor for converting the female number to the male number and published all numbers on the male standard. Without exception each generation produced successively higher

[1] Contribution from the Zoölogical Laboratory of the University of Illinois, No. 97.

numbers in the upward selected lines and successively lower numbers in the downward selected lines, and in each case the three lines were very close together. The average facet number was raised from 98.0 to 139.5 and lowered from 98.0 to 83.7. The maximum was raised from 182 to 213 and the minimum was lowered from 45 to 36. This increase in the range was not due to a larger number of counts, as 500 flies were counted from the stock and only 450 from the selected lines.

The Experiments of MacDowell.

In the same year E. Carlton MacDowell published the results of selections for higher bristle numbers in a race of *Drosophila* with extra bristles on the thorax. For six generations the number of extra bristles increased, but failed to rise any higher in forty additional generations. The response to selection was not so definite as in the experiments of Zeleny and Mattoon. The variation in the averages from different lines was very large. While the average of a large number of lines increased with each generation, that of any particular line often decreased for a generation. This wide range of fluctuations in the averages from different lines was partly explained by the existence of a correlation between the number of extra bristles and the size of the fly.

There is, however, another possible cause that was apparently overlooked. MacDowell selected for extra bristles in two arbitrarily limited rows on the dorsal side of the thorax, but states that even when no extra bristles were present in those rows "these flies frequently showed extra bristles on other parts of the thorax." The factors for extra bristles evidently controlled not only the number of extra bristles in those rows, but also the number of extra bristles on other parts of the thorax and possibly over the entire body. Selection, then, was made for only a small part of a variable character. A high number of bristles in the area under observation would in general indicate a high number of bristles on other parts of the body, but might actually be accompanied by a low number of extras elsewhere. Low offspring from high parents could be accounted for in that way. The efficiency of the selection would also

decrease as the part of the character under observation decreased in comparison with the part not under observation.

The Present Experiments.

Since the experiments of Zeleny and Mattoon gave such clear results, but were interrupted before they led to a final conclusion, it seemed desirable to repeat them on a larger scale and continue them for a greater number of generations to determine if a pure line could be established and to study the changes in the existing factor or factor complex.

Material and Methods.

In order to have a check on any possible contamination a stock was selected which had a second recessive character, the vestigial wing, in addition to the bar eye. This stock also had the advantage of having on the average a lower facet number than the long-winged stock. But sterility in the race and the indefinite character of the results made it necessary, after a few generations, to return to the long-winged stock. In both cases the eye color was that of the normal wild fly.

The vestigial-winged, bar-eyed stock was designated as VBa; the long-winged, bar-eyed stock as Ba. The downward and upward selected lines in VBa were distinguished by l and h, in Ba by d and u respectively. Individual lines were distinguished by numbers. Any given mating received the number of the generation to which it belonged and a serial number corresponding to the number of matings made from that line in the given generation. In that way a number like Bau4f2–5, although cumbersome, gives nearly the whole pedigree of the mating involved. Since the lines are now clearly distinguished without the stock designations, Ba and VBa, those designations will frequently be omitted in this paper.

The material for the present set of experiments was obtained from Professor Charles Zeleny on whose advice the work was undertaken. To him the author is also indebted for a keen interest in the work and for many helpful suggestions.

All stocks were kept in large bottles while eight dram vials were used for individual pairs. The vials were plugged with

cotton and in case of the long-winged race contained pieces of filter paper to prevent the flies from adhering to the food.

Throughout these experiments bananas were used as food. Only specimens with perfect skin were selected. The pulp was never allowed to come in contact with the outside of the skin

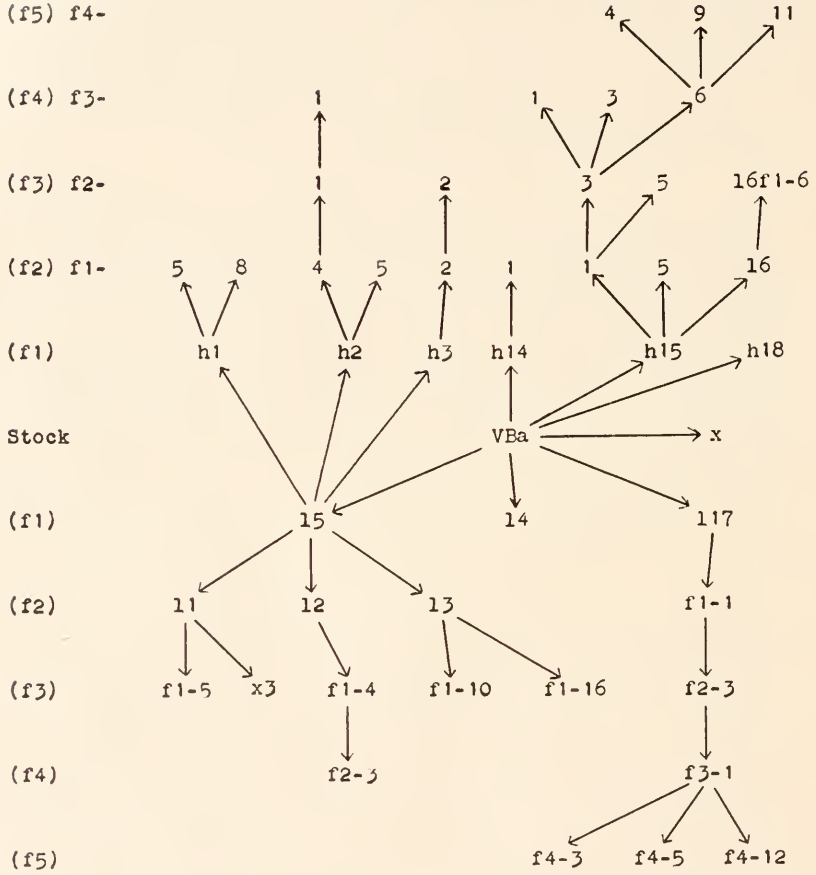

FIG. 1. Relation of selected lines and stock in VBa. In general the downward selected lines are below and the upward selected lines above.

and was heated to 70° C. and allowed to cool in a closed vessel before being placed into the food jar. Food was allowed to undergo alcoholic fermentation for about three days before being used. At first the author had considerable difficulty in keeping food, as acetic acid fermentation soon set in, the food

became hard, and larvæ did not seem to thrive well in it. This defect, however, was remedied after one or two generations had been reared from the vestigeal-winged stock and long before the experiments on the long-winged stock were undertaken.

In some preliminary work the number of facets was estimated in the eyes of the parents selected and the actual counts were made at the time the parents were killed. This method was employed by Zeleny and Mattoon. The author, however, found that his estimates were not close enough and that in some cases parents were lost because they died and were destroyed by the larvæ before their death was discovered. The facets of dead flies also disintegrate when they remain in contact with food for a short time. For those reasons all selections during the experiments were made from actual counts.

During all of the work on the vestigial-winged flies and during part of the work on the long-winged flies, selections were made at noon and night after all the flies had been removed in the morning. In that way the specimens were never more than six hours old at the time of selection. But observations during that time showed that males seldom or never mated before they were twenty-four hours old, and for that reason later selections were made every twelve hours. All desirable specimens were saved and when no mates were present they were kept in vials with food until matings could be made.

All flies were etherized and the facets were counted while they were quiescent. The larvæ were never subjected to ether, as the flies to be examined were first transferred to empty vials. Selected flies recovered from the effect of the ether in fifteen minutes to half an hour. The other flies were preserved in 85 per cent alcohol.

For counting facets the flies were placed in a little pit on a paraffin block and illuminated by means of a 25-watt tungsten lamp. This gave sufficient light but only a moderate amount of heat. A Leitz microscope with a number four ocular and a number three objective and the tube drawn out to its full length was found to be most convenient. This gave a relatively high magnification with a suitable depth and size of field.

Errors in counting can not be avoided; it is merely a question

of reducing them to a minimum. In eyes with 100 facets or less the error is certainly less than one per cent., but it increases rapidly as the facet number increases. Recounts made on eyes

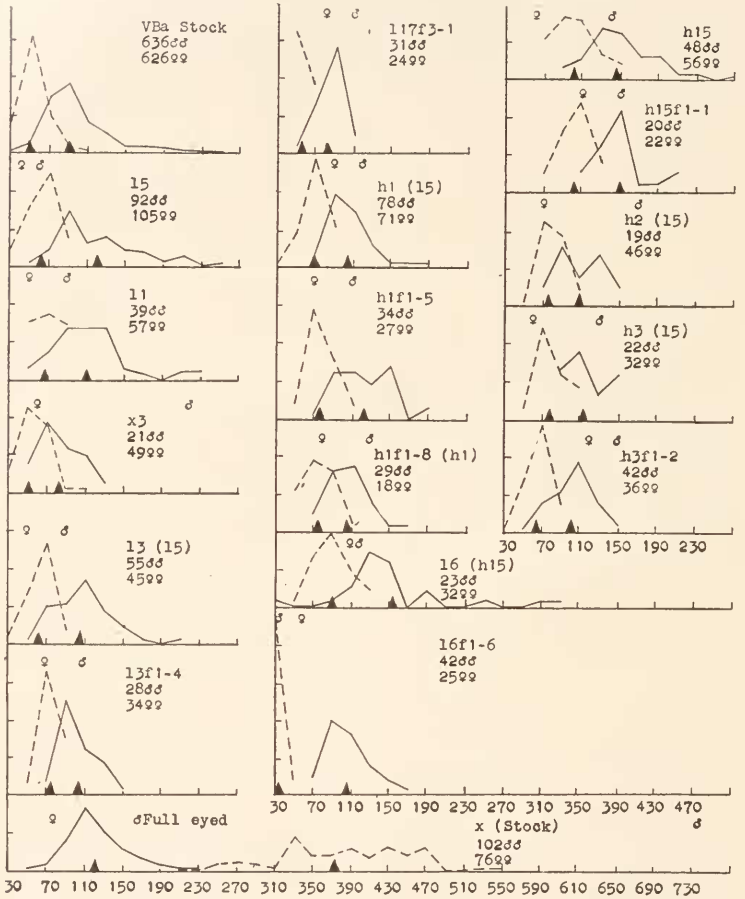

FIG. 2. Curves showing the effect of selection in individual lines in VBa. All curves are plotted on the scale of fifty. The males are represented by continuous lines and the females by interrupted lines. The parents are indicated at the top and the averages by the black pyramids below. The values plotted are for flies between 20 and 39, 40 and 59, 60 and 79, etc. The number of the mating is given above, followed by the source in brackets unless the parents come from the mating represented by the curve just above. In case of difficulty in determining the relationship of the lines consult Fig. 1.

with as many as 300 facets showed an error of possibly two or three per cent. Of course it takes two or three weeks of practice to reach that point of efficiency.

Errors are due chiefly to the following causes: (1) The arrangement of the facets may be irregular. Ordinarily the facets are arranged in rows and one can easily count two rows at a time even if the eye is large. But in the bar eye the dorsal part usually contains a large number of facets without any indication of rows. This area increases very rapidly with the size of the eye. In heterozygous females the facets are all arranged in beautiful rows, and one is thus able to distinguish readily between the eye of a heterozygous female and a bar-eyed male even if both eyes contain the same number of facets. Bar-eyed females as a rule contain more regular facets than the males and the distinction between low heterozygous females and high bar-eyed females is more difficult. (2) There may be small facets either among the others or at the margins. These facets are most abundant in irregular eyes and may be one of the causes of irregularity. All grades are found from mere prominences that can only be seen with the most advantageous light to normal-sized facets. As a rule, however, there are few if any doubtful facets. (3) There may be colorless facets at the margins. Normally the colored area of the eye extends somewhat beyond the facet area, but in some rare cases a few facets may extend beyond it and these may be overlooked. (4) When the eye is so large that the fly must be turned to count all of the facets some of the facets in the middle may be recounted or omitted. All heterozygous females and some high grade males had to be turned. The error in this case also is not very great, as the bar eye as well as the heterozygous eye is almost divided in the middle. (5) Errors may also arise through a lack of concentration or through the inability to make the eye retain its position. These are purely personal elements.

No mechanical device was used for marking off the counted area from the uncounted area on the eye of the fly. The author relied entirely on the ability of his eye to follow the rows or, in the absence of rows, to mark off certain areas and hold them until the eye was counted. A cross-hair in the ocular was tried, but was found to be unreliable. It could be used if the eye of the fly presented a flat field, but with the rounded contour of the eye the hair keeps on traveling over facets as one focuses up

and down and comes to rest on the same facets only in the same focal plane. But it is impossible to count all facets without large changes in the focus. In shifting the fly after a given area has been counted it is evident that the same shift makes the hair pass over more facets on a slanting area than on a horizontal one, just as surveyors will pass over more surface in surveying up or down a hill than on the level. But on the rounded eye the hair usually passes over both a horizontal and a slanting area at the same time. For that reason one can not possibly shift so that the hair crosses the same number of facets at every point on the eye. A cross-ruled ocular has little or no advantage over a cross-hair, and a camera lucida is scarcely worthy of consideration as it is difficult enough to keep it at the proper place even on a perfectly flat field.

The largest error, by far, is due to the fact that only the right eye was counted. Zeleny and Mattoon reported that they found the averages of a large number of counts to be the same for both eyes. The author obtained the same results. But that does not mean that both eyes in any given fly have the same number. Normally the variation is not more than about 1 per cent from the mean, but the author has found it as high as 5 per cent, and in one case 10 per cent. One abnormal male was obtained with 25 facets in the right eye and 146 in the left. It is obvious that the error is greatest in the parents and the extremes of the offspring as we are dealing in those cases with individual flies. In parents the left eye was usually examined to see that it was not abnormal, and actual counts were made in a large number of cases. The abnormal male was mated to see if the unequal condition would be inherited, but it died within twenty-four hours without giving any offspring.

Selections in the Vestigial-Winged Stock.

When the bottle that was to give rise to the VBa stock was received, flies were transferred to two fresh stock bottles and the offspring from these parents were used for making the original selections. From October 31 to November 5 some preliminary counts were made on flies that hatched in the original bottle. Sixty-nine males gave a mean facet number of 115 and seventy females gave a mean of 63.

All of the offspring from the parents placed into the new stock bottles were counted, including the ones that were used to continue the stock, and selections were made during the entire period. From the preliminary counts it was concluded that flies above a certain limit could be selected as high and those below another limit as low; but when the selections were begun it was found that no flies appeared that approached the lower limit set for high selections and many flies were below the limit set for low selections. As a result a new standard had to be established. Later the stock became more variable and some higher flies appeared together with some very low ones; indeed the lowest female obtained during these experiments was taken from one of these bottles. In all 370 males gave an average of 96 facets and 364 females gave an average of 54 facets. More counts on the stock were made March 17 to April 2, and this time 197 males gave an average of 82 facets and 192 females, an average of 44. This variability of the stock was contrary to the conditions found by Zeleny and Mattoon and seriously interfered with the success of the present experiments. It was necessary to pay as much attention to the nature and possible cause of the variation as to the effect of selection.

The greatest difficulty encountered in the VBa selections, however, was sterility and low production in the single lines. Of 42 matings made from the stock only 6 were fertile and of these only 2 gave a sufficient number of offspring for selection. On the whole about 80 per cent of the matings were sterile, and in many cases even lot matings failed to produce any offspring. By the end of the fifth generation all lines had died out except l17 and h15, and these were saved only through lots. For that reason the experiment was discontinued at that point.

No special experiments were made to determine the cause of sterility. Obviously it was not due to inbreeding, for it was as great in the first generation as in succeeding ones. In the majority of cases it was also not due to the inability of the females to deposit eggs as was the case in the experiments of Hyde. A few of such abnormal females were observed, but in most cases eggs appeared in the food and failed to hatch. The

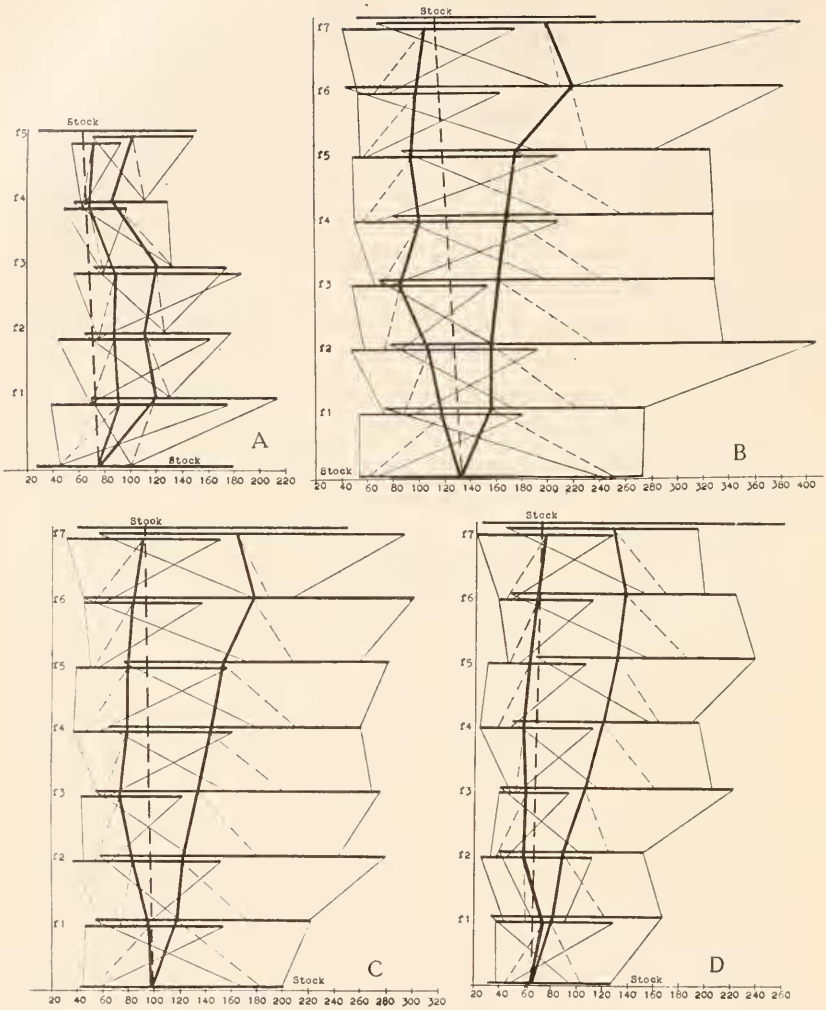

FIG. 3. The effect of selection on range and average. A, high and low lines in VBa; B, males in Ba, high and low lines; C, high and low lines in Ba; D, females in Ba, high and low lines. A and C are plotted for the means between male and female averages. These figures do not represent individual lines, but include all lines except reverse selections for the generations indicated. The heavy horizontal lines indicate the range, the heavy vertical lines, the averages. The heavy interrupted vertical lines merely connect the initial and final stock averages and do not indicate the averages of the stock at intermediate points. The fine slanting lines connect the extremes of the parents with those of the offspring while the fine interrupted lines connect the averages of the parents with those of the offspring. The facet numbers are indicated at the bottom, the generations at the left hand side.

results of Castle correspond more nearly to the facts observed.

The results obtained from these selections can best be made out by consulting Table I. and Figs. 1, 2 and 3.

The attempt to select downward was entirely unsuccessful. The selection was never very rigid, but the average of the offspring persistently remained above that of the stock.

The upward selections were more successful. In h15 the males rose from the stock average of 96 to 147 and the females from 54 to 101. In the other two lines where smaller numbers of offspring were obtained the rise was not quite so large. The second and third generations, however, failed to give any further increase in the facet number. Upward selections taken from line l5 after the first generation failed to give any rise in the facet number. The line x3 was taken from l1 after two generations of downward selection and gave a decrease in the facet number in spite of the fact that the male had 222 facets and was mated to average females.

The line l6 was intended to be a downward selection from h15, but the parents proved to be very near the mean and all matings from the first generation were sterile except f1–6. This particular mating involved a male with 34 facets, the lowest number obtained in these experiments. Fortunately a fair number of offspring was obtained. The males average much lower than those of the previous generation and about the same as those of the low lines hatching at the same time, but the females average much lower than anything obtained elsewhere. It is likely that the male with 34 facets was a mutant and that the mutation chiefly concerned the sex chromosome. The complete sterility of this race prevented any further investigation.

The results here obtained are very different from those reported by Zeleny and Mattoon. In their selections all three generations went gradually upward and gradually downward; in these experiments there was a sudden rise in the upward selected lines in the first generation and no further effect, and no response at all in the downward selected lines. For that reason and also on account of the high degree of sterility it seemed best to make some selections in the long-winged stock used by Zeleny and Mattoon.

Table I.

Relation of Parents and Offspring. Vestigial-Winged, Bar-Eyed Stock (VBa).

Parents.				Offspring.							
				♂				♀			
Bottle Number.	Source.	Facets.		No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
Stock	Stock			636	93.8	253	36	626	51.9	108	19
l4	Stock	63	46	3	101.7	134	85	12	71.9	93	38
l5	Stock	60	45	92	120.2	254	56	105	63.2	98	23
l1	l5	86	48	39	110.3	222	57	46	67.4	95	41
l1f2	l1	64	46	10	116.5	269	77	12	73.8	104	56
l2	l5	56	37	13	107.3	142	70	14	69.2	88	52
l3	l5	98	53	55	106.3	208	56	45	63.6	88	32
l3f1–4	l3	108	71	28	102.9	149	76	34	74.7	97	51
l3f1–10	l3	96	53	9	116.1	142	82	10	77.6	96	56
l3f1–16	l3	75	56	1	121.0			1	86.0		
l3f2–3	l3f1–4	78	51	2	57.0	58	56	5	50.2	54	47
l17	Stock	46	19	2	98.0	108	88	2	85.5	90	81
l17f2	l17	108	90	4	118.8	122	116	8	92.4	102	83
l17f2–3	l17f2	116	83	12	100.9	165	70	17	60.1	89	42
l17f3–1	l17f2–3	113	81	31	85.0	117	57	24	56.3	76	41
l17f4–3	l17f3–1	77	54	9	84.2	96	70	3	64.0	74	51
l17f4–5	l17f3–1	lot	lot	16	82.4	108	60	11	57.2	77	48
l17f4–12	l17f3–1	lot	lot	2	90.0	100	80	6	59.8	65	53
h1	l5	120	86	78	106.2	182	71	92	70.1	99	29
h1f1–5	h1	108	74	34	122.0	192	78	27	77.7	101	58
h1f1–8	h1	128	75	29	107.3	160	75	18	73.6	103	52
h2	l5	170	73	19	110.7	163	68	46	79.4	105	53
h2f2	h2	156	90	5	146.2	175	125	6	89.3	113	79
h2f1–5	h2	163	100	4	88.0	112	72	6	58.7	69	48
h2f2–1	h2f2	150	113	2	103.5	115	92	6	59.7	70	50
h2f3–1	h2f2–1	115	70	14	109.6	143	88	13	67.4	89	55
h3	l5	126	64	22	114.8	154	82	32	80.0	116	57
h3f2	h3	145	116	42	102.7	150	52	36	67.4	95	29
h3f2–2	h3f2	122	90	3	80.3	85	72	1	65.0		
h14	Stock	155	64	9	128.3	190	95	3	89.0	108	77
h14f2	h14	161	108	4	94.0	113	74	9	69.0	82	63
h15	Stock	137	60	48	147.1	271	81	56	101.2	158	70
h15f1–5	h15	123	96	17	121.7	154	84	14	83.6	113	56
h15f2	h15	148	112	20	149.7	219	105	22	102.5	137	64
h15f2–3	h15f2	lot	lot	16	147.2	199	92	19	91.4	125	51
h15f2–5	h15f2	205	111	16	151.6	217	118	8	94.0	129	57
h15f3–1	h15f2–3	156	109	10	96.7	126	76	8	73.5	123	59

Parents.				Offspring.							
Bottle Number.	Source.	Facets.		♂				♀			
				No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
h15f3–3	h15f2–3	150	107	3	84.0	97	62	3	76.7	85	61
h15f3–6	h15f2–3	158	102	9	97.0	134	63	19	73.8	103	50
h15f4–4	h15f3–6	131	103	7	120.6	196	79	4	89.3	100	65
h15f4–9	h15f3–6	lot	lot	3	117.3	144	88	3	78.7	87	63
h15f4–11	h15f3–6	134	93	2	124.0	131	117	3	74.3	86	63
h18	Stock	163	62	12	123.8	241	90	11	94.8	129	59
l6	h15	122	108	23	151.3	338	34	32	89.6	127	56
l6f1–6	l6	34	64	42	105.1	170	70	25	33.5	56	25
x	Stock	full	43	102	122.3	228	56	76	377.9	543	218
x3	l1	222	lot	21	82.0	127	56	49	54.9	104	31
x4	l6	328	lot	12	142.0	243	70	16	77.4	116	53
x4f1–1	x4	140	116	5	146.0	181	130	10	111.5	135	83
x4f2–5	x4f1–1	132	103	6	169.5	196	143	1	119.0		

Selections in the Long-Winged Stock.

These experiments were begun on January 15 and continued until the middle of June. In this case the number of flies counted from the stock during selection was 91 males with an average facet number of 132 and 81 females with an average of 66. The first selection made was very near the mean and was designated as Ba1 and used as part of the stock. It yielded 44 males and 37 females with mean facet numbers of 125 and 68 respectively. In June the eyes of 73 additional males and 97 additional females from the stock were counted, yielding mean facet numbers of 114 and 72 respectively. Here again, as in the vestigial-winged stock, there is a change in the mean facet number, and in this case it also involves a decided change in the ratio between male and female facet numbers.

Little difficulty was encountered on account of sterility in this stock. About 75 per cent of the matings were fertile, and nearly 75 per cent of the fertile matings produced 50 offspring or more. In each case all of the offspring were counted. To avoid the dying out of lines a number of branches of each line was run, only brothers and sisters were mated, but no attention was paid to the dying out of any given branch when it did not

come up to the standard in facet number or production. In that way the most suitable flies from the most suitable branches were selected, bringing the selections to some extent on the behavior basis. By that method parents whose somatic constitution varies from their germinal constitution are to some extent eliminated.

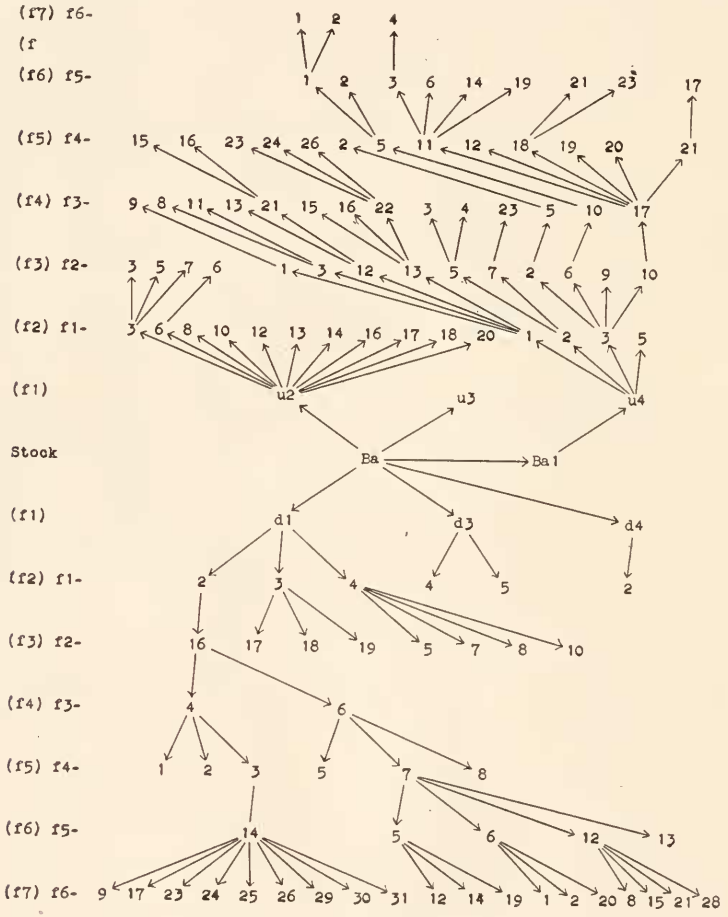

FIG. 4. Relation of selected lines and stock in Ba.

Selections in this stock were carried on for seven generations in each direction, several return selections were made, and crosses in both directions were finally made from the selected lines. The results are shown in Table II. and in Figs. 3, 4, 5, 6 and 7.

The downward selections were not very effective. In the first generation the average facet number in the females actually rose above that of the stock, but the average of the males dropped

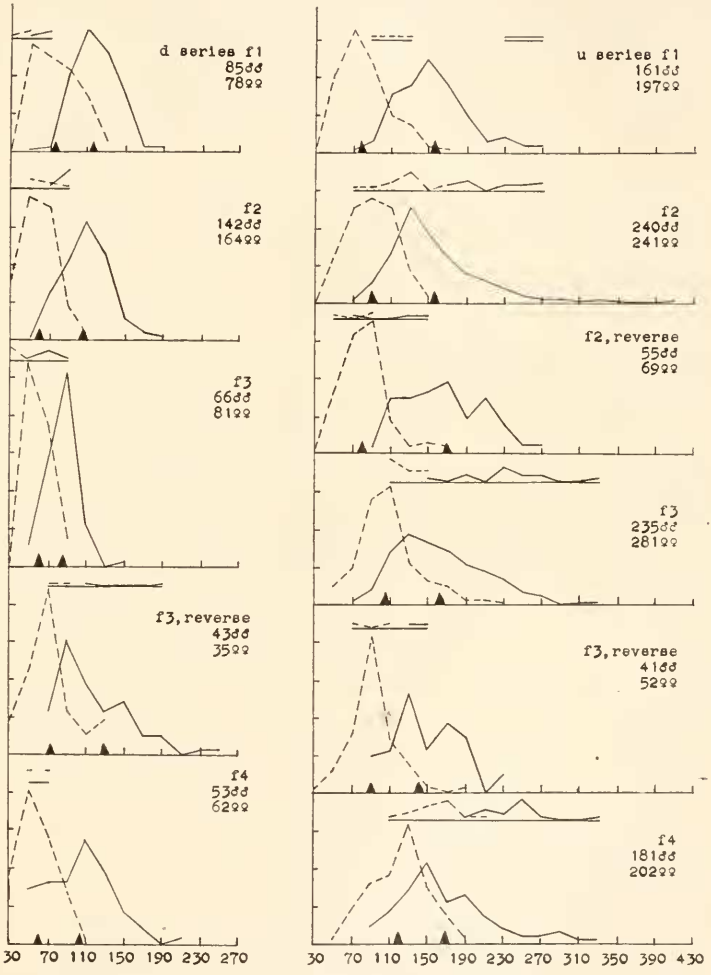

FIG. 5A.

considerably below. In the next generation the females dropped decidedly and the males dropped only slightly. The third generation produced another slight lowering of the means, and after that there was a gradual return toward the mean of the stock.

In the seventh generation the mean of the stock had been reached by the females, but the males were still slightly lower. Taking the mean of the male and female averages more uniform results appear to have been obtained. In that case the first generation shows no change, the second and third generations show a gradual

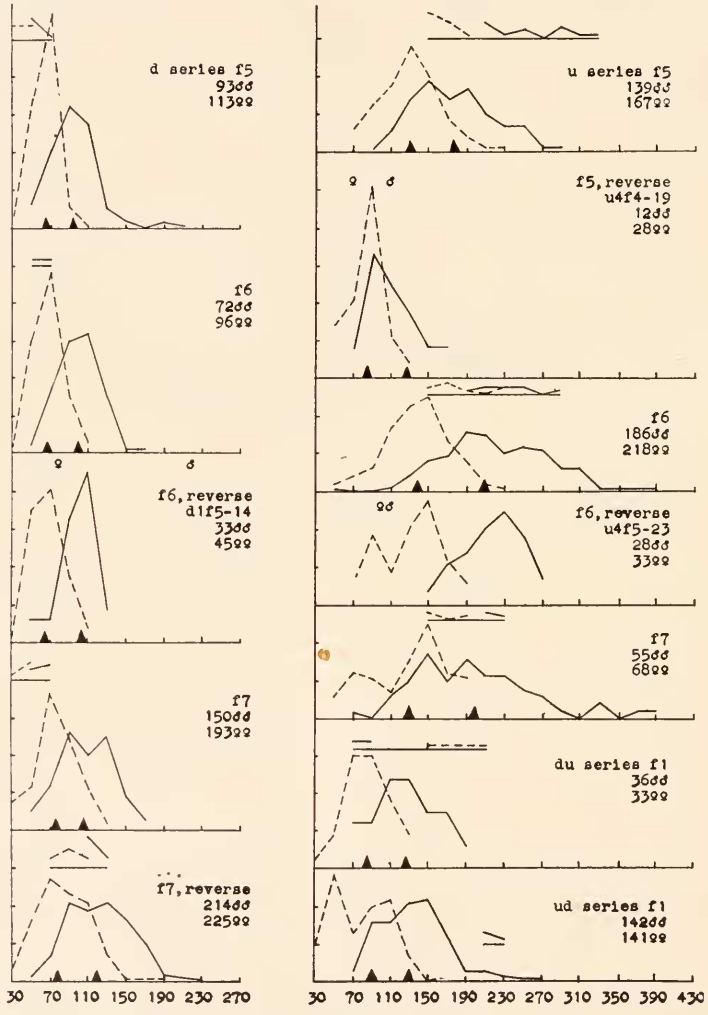

FIG. 5B.

FIG. 5. Curves showing the effect of selection for generations in Ba. These curves are plotted on a scale of one hundred. Parents are indicated by smaller curves at the top. In other respects the curves are like those of Fig. 2.

lowering, but the next four generations show a complete return to the mean of the stock.

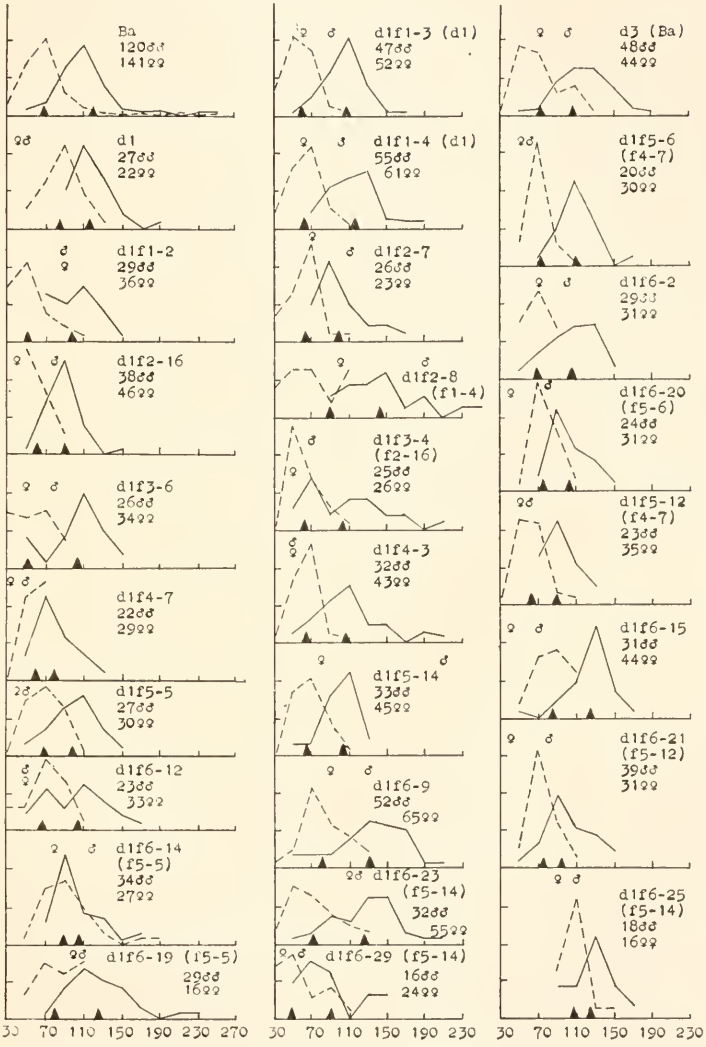

FIG. 6. Curves showing the effect of selection in individual lines in Ba, downward selections. For other information consult Fig. 2.

The upward selections were more successful. With the exception of the last generation there is in every case a slight rise

in both sexes. The results in the last generation can be explained by the fact that the selections were below the average

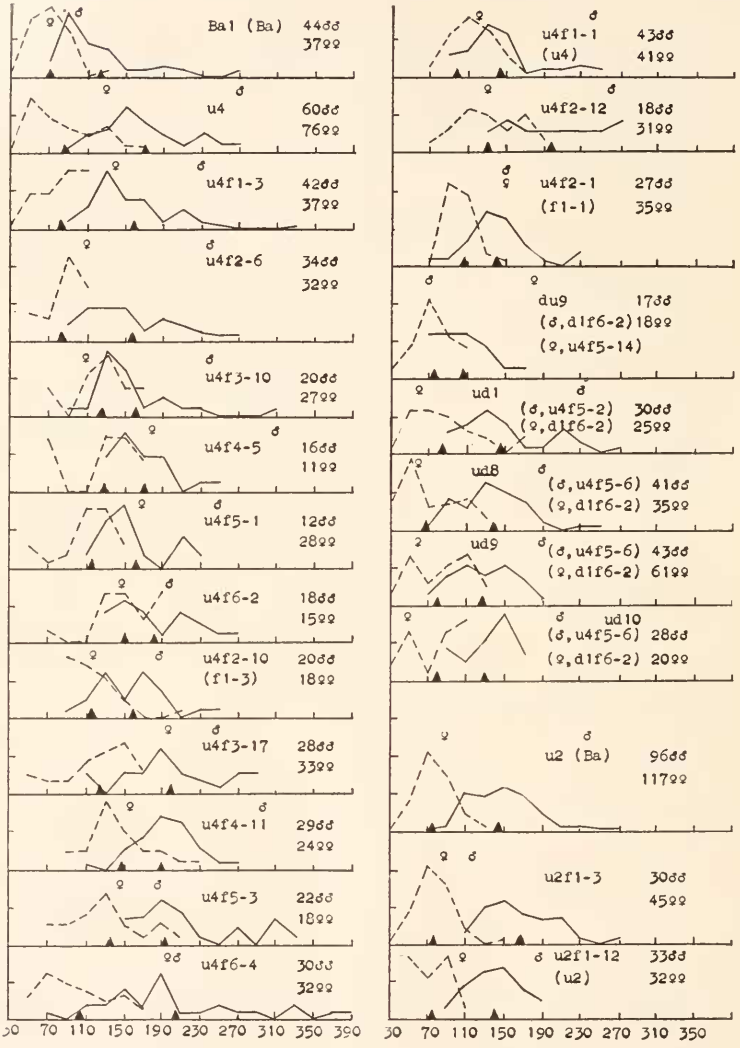

FIG. 7. Curves showing the effect of selection in individual lines in Ba, upward selections and crosses. For other information consult the description of Fig. 2.

in the males and not far above in the females, and the fact that the offspring hatched at a time when all counts were low. This will be explained later.

TABLE II.

RELATION OF PARENTS AND OFFSPRING. LONG-WINGED, BAR-EYED STOCK (Ba).

Parents.				Offspring.							
				♂				♀			
Bottle Number.	Source.	Facets.		No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
Ba	Stock			120	122.7	240	53	141	69.1	259	24
Ba1	Stock	104	74	44	125.0	273	69	37	68.3	128	34
d1	Stock	53	38	27	117.4	180	91	22	84.0	122	56
d1f1–2	d1	92	91	29	99.5	141	64	36	52.5	102	25
3	d1	92	56	47	106.0	164	58	52	58.4	108	28
4	d1	98	65	55	114.7	192	62	61	59.4	111	32
d1f2–5	d1f1–4	84	62	0				1	65.0		
7	4	106	73	26	97.9	162	62	23	62.6	117	37
8	4	190	98	17	148.6	242	85	12	90.0	131	47
10	4	87	47	11	87.1	104	67	11	63.4	87	48
16	2	76	36	38	87.6	153	58	46	61.6	92	40
17	3	75	36	12	72.3	95	49	17	54.5	71	38
18	3	68	35	4	80.0	87	73	6	63.7	73	58
19	3	58	33	1	109.0			1	64.0		
d1f3–1	d1f2–10	76	48	2	88.5	90	87	2	50.0	53	47
4	16	68	54	25	103.2	209	51	26	60.6	111	26
6	16	79	53	26	101.8	154	57	34	55.1	98	23
d1f4–1	d1f3–4	57	48	11	98.9	122	74	7	64.6	68	59
2	4	51	44	11	90.2	107	79	13	70.2	88	49
3	4	55	47	32	109.4	208	55	43	62.2	104	37
5	6	59	32	4	87.0	96	75	6	66.8	82	37
7	6	57	23	22	80.3	133	56	29	59.1	77	29
8	6	69	35	13	91.2	115	50	15	63.5	91	49
d1f5–5	d1f4–7	56	45	27	98.9	155	54	30	70.8	100	37
6	7	61	46	20	111.5	165	78	30	72.3	105	45
12	7	56	55	23	90.2	127	65	35	61.3	110	39
13	7	62	51	2	78.5	83	74	1	71.0		
14	3	208	77	33	101.1	132	56	45	66.6	116	30
d1f6–1	d1f5–6	165	105					1	67.0		
2	6	102	70	29	108.2	155	59	31	69.7	99	42
8	12	126	110	2	102.5	103	102	3	85.0	90	81
9	14	125	93	52	131.5	215	50	65	82.0	139	27
12	5	54	49	23	103.7	166	42	33	69.3	104	22
14	5	122	83	34	103.3	164	69	27	93.8	187	52
15	12	68	45	31	123.4	167	51	44	86.1	114	44
17	14	119	92	1	121.0						
19	5	111	100	29	127.3	237	71	16	84.6	117	52
20	6	78	45	24	101.9	144	72	31	78.9	112	49
21	12	78	39	39	97.4	153	51	31	72.7	104	40
23	14	116	110	32	125.3	206	51	55	70.9	135	23

TABLE II.—*Continued.*

Bottle Number.	Parents. Source.	Parents. Facets. ♂	Parents. Facets. ♀	Offspring. ♂ No. Flies.	♂ Facets. Ave.	♂ Facets. Max.	♂ Facets. Min.	Offspring. ♀ No. Flies.	♀ Facets. Ave.	♀ Facets. Max.	♀ Facets. Min.
d1f6–24	14	114	94	13	135.5	185	81	16	104.2	127	73
25	14	113	90	18	125.1	163	89	16	107.5	145	86
26	14	108	66	20	105.0	147	51	7	87.3	121	49
28	12	77	48	14	127.1	176	72	24	88.0	125	30
29	14	57	43	16	90.8	158	56	24	53.9	109	20
30	14	117	72	13	115.5	178	78	19	65.8	105	42
31	14	56	30	3	88.3	95	83	6	71.2	90	46
d3	Stock	65	51	48	118.2	181	54	44	72.8	128	37
d3f1–4	d3	74	43	1	92.0			7	72.0	92	54
5	d3	84	45	2	59.0	70	48	1	45.0		
d4	Ba1	69	48	10	116.3	145	81	12	66.9	86	50
d4f1–2	d4	90	61	8	128.6	157	108	5	64.2	78	54
u2	Stock	238	94	96	147.6	268	74	117	77.5	132	39
u2f1–3	u2	122	89	30	165.7	261	103	45	75.2	141	32
6	u2	144	94	15	187.7	249	107	14	90.9	162	52
8	u2	172	92	28	172.1	408	79	26	90.2	148	59
10	u2	187	132	22	191.8	330	125	14	104.1	144	69
12	u2	190	111	33	141.6	191	86	32	74.7	113	43
13	u2	192	109	16	144.6	203	90	18	94.4	135	49
14	u2	220	110	2	175.5	186	165	4	116.5	130	105
16	u2	246	123	8	126.3	153	106	10	95.1	111	75
17	u2	268	126	5	206.6	278	130	4	116.3	124	110
18	u2	172	63	22	141.7	249	78	38	81.0	138	49
20	u2	74	56	10	127.1	221	90	10	90.5	100	77
u2f2–3	u2f1–3	190	102	15	157.0	240	88	16	91.3	116	72
5	3	261	146	1	136.0			2	121.5	134	109
6	6	220	114	4	114.0	120	104	2	81.0	88	74
7	3	136	76	6	107.7	138	81	6	77.0	88	68
u3	Stock	240	109	5	124.8	138	98	4	101.8	120	93
u4	Ba1	273	128	60	170.9	275	99	76	85.3	167	34
u4f1–1	u4	241	121	43	146.3	255	83	41	94.5	151	49
2	u4	271	160	18	154.2	274	100	15	105.5	140	66
3	u4	227	136	42	158.2	336	96	37	83.1	116	39
5	u4	275	167	1	161.0			2	100.5	118	83
u4f2–1	u4f1–1	153	151	27	141.4	231	74	35	106.0	173	61
2	3	147	106	35	153.3	221	93	46	92.1	191	35
3	1	225	116	51	151.6	331	70	41	87.3	144	40
5	2	223	140	13	179.4	224	112	11	110.0	173	51

TABLE II.—*Continued.*

Bottle Number.	Parents. Source.	Parents. Facets. ♂	Parents. Facets. ♀	Offspring. ♂ No. Flies.	Offspring. ♂ Facets. Ave.	Offspring. ♂ Facets. Max.	Offspring. ♂ Facets. Min.	Offspring. ♀ No. Flies.	Offspring. ♀ Facets. Ave.	Offspring. ♀ Facets. Max.	Offspring. ♀ Facets. Min.
u4f2–6	3	237	111	34	156.9	263	83	32	86.4	113	40
7	2	274	129	31	166.1	238	107	54	109.9	217	45
9	3	336	100	8	179.1	229	150	11	108.3	169	85
10	3	187	118	20	156.8	244	99	18	114.8	205	81
12	1	255	127	18	199.1	266	126	31	131.0	190	66
13	1	252	122	13	203.4	267	119	28	126.3	221	69
u4f3–3	u4f2–5	215	173	3	135.7	165	99	6	147.0	193	84
4	5	213	147	7	160.4	182	125	14	124.9	166	61
5	2	197	121	30	173.1	330	92	23	112.1	154	75
8	3	331	173	7	142.9	198	108	2	151.5	166	137
9	1	231	143	3	148.0	190	117	6	124.7	158	113
10	6	241	112	20	159.2	308	99	27	126.5	176	64
11	3	242	136	8	156.5	207	129	7	115.3	127	96
13	12	260	190	12	194.7	291	140	14	126.2	150	89
15	13	245	172	4	149.5	185	113	14	114.6	148	83
16	13	221	172	9	139.3	167	112	6	102.0	136	70
17	10	244	205	28	201.0	298	108	33	122.2	168	48
21	12	255	179	18	175.5	294	132	16	122.0	167	65
22	13	255	145	15	178.9	244	99	25	115.6	190	67
23	7	216	145	17	127.1	281	82	9	94.3	149	75
u4f4–2	u4f3–5	330	154	22	159.3	260	88	20	99.7	141	67
5	10	308	176	16	169.9	240	129	11	125.6	173	69
11	17	290	157	29	191.6	269	101	24	145.3	230	81
12	17	298	158	5	144.4	168	120	4	129.5	141	125
15	21	203	156	2	165.5	167	164	7	114.0	137	77
16	21	220	165	4	155.0	187	138	4	138.5	158	129
18	17	284	156	15	190.6	284	106	27	134.2	237	77
19	17	109	67	12	128.9	192	77	28	84.1	127	52
20	17	250	155	3	153.7	196	109	6	120.2	141	86
21	17	209	152	11	186.0	256	132	8	141.1	195	84
23	22	214	163	13	173.7	257	125	17	123.2	160	78
24	22	244	190	17	186.8	327	130	37	136.1	182	85
26	22	209	161	2	120.0	121	119	2	131.5	135	128
u4f5–1	u4f4–5	238	173	12	158.3	222	111	28	113.3	153	46
2	5	240	167	14	182.2	235	118	10	135.3	162	76
3	11	189	153	22	195.6	302	123	18	133.9	209	76
6	11	213	151	42	225.2	384	45	37	127.6	177	65
14	11	249	182	43	247.0	367	143	50	137.7	198	72
17	21	205	162	31	242.2	354	151	28	148.5	201	58
19	11	231	236	14	215.7	303	140	40	151.0	221	92
21	18	284	237	8	192.5	210	170	7	151.3	185	103
23	18	106	98	28	217.2	278	151	33	130.4	186	64
u4f6–1	u4f5–1	222	153	7	213.3	260	116	21	152.2	191	81
2	1	201	149	18	184.1	262	128	15	149.3	191	76
4	3	209	196	30	204.4	398	69	32	99.1	174	43

TABLE II.—*Concluded.*

Bottle Number.	Parents. Source.	Facets. ♂	Facets. ♀	Offspring. ♂ No. Flies.	♂ Facets. Ave.	♂ Facets. Max.	♂ Facets. Min.	Offspring. ♀ No. Flies.	♀ Facets. Ave.	♀ Facets. Max.	♀ Facets. Min.
du1	6–2 4–11	67	200	12	148.3	198	116	9	98.0	122	67
2	5–12 5–2	82	151	6	146.8	178	110	5	95.4	124	60
8	6–2 5–3	88	171	1	135.0			1	112.0		
9	6–2 5–14	74	182	17	104.6	160	66	18	73.5	113	39
ud1	5–2 6–2	235	59	30	149.1	264	81	25	83.8	166	32
8	5–6 6–2	205	56	41	138.2	245	61	35	69.1	123	31
9	5–6 6–2	205	56	43	127.1	187	72	61	81.3	129	30
10	5–6 6–2	211	54	28	132.0	169	85	20	80.1	113	28

TABLE III.

DAILY COUNTS, CROSSES BETWEEN LOW MALES AND HIGH FEMALES.
(Males from d1, Females from u4.)

Date.	Badu1. ♂ Bot. 1.	Badu1. ♂ Bot. 2.	Badu1. ♀ Bot. 1.	Badu1. ♀ Bot. 2.	Badu2. ♂ Bot. 1.	Badu2. ♂ Bot. 2.	Badu2. ♀ Bot. 1.	Badu2. ♀ Bot. 2.	Badu8. ♂	Badu8. ♀	Badu9. ♂ Bot. 1.	Badu9. ♂ Bot. 2.	Badu9. ♀ Bot. 1.	Badu9. ♀ Bot. 2.
My 24					117		124 110							
25	198 133 147				149 110 166									
26	163		122		178					112				
27	157 186	134	121 99		161				135		136 113		100 84 89	
Je 6	131 117 158 139 116		76 67 114 97	89 97				60 97 86			148 134 110 160 99 111 102 95 123 93	67 82 68 71 66	39 113 86 69 60 92 63 54 61 52 69 103 70 70	49

TABLE IV.

DAILY COUNTS, CROSSES BETWEEN HIGH MALES AND LOW FEMALES.
(Males from u4, Females from d1.)

Date.	Baud1.				Baud8.				Baud9.				Baud10.	
	♂		♀		♂		♀		♂		♀		♂	♀
	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.		
My 24	159 148													
25	200 238 264 224		133 161		144 138		121 112 116		158 121		104 126 98 115 113			
26	164 211 203		166 118 123		163 125 133 153 143 136 136		119 123 104		149 179 187 175 174 144 116		116 96 120 113 98 119 105 109 127		152 169 168 120 127 157 133	93 102 96
27	133 184 136 132 205 129		106 105 71 82 80		177 169 155 245 138 198 142 153		89		172 169 183 178 153 153		111 122 98 116 112 111 113 80 108 129 124		161 161 144 131 146 155 149 146	93 113 106 113 112 112 113
Je 7, 8	104 81 126 94 154 97 136	147 114 133 108 141 118 101 89	84 54 83 65 72 54	58 64 99 32 40 52 56 63 73	166 239 158 111 151 163 96 83	92 123 107 96 170 108 98 125 114 Full 134 126 61 94 95 114 195	49 57 57 51 77 46 47 88 87	34 80 76 62 45 77 54 46 52 41 34 31 48 104 87 37 32 36 100	102 150 140 128 91 109 72 81 127 92 121 150	97 72 116 85 135 118 76 113 121 103 105 83 111 141 92 123	66 33 54 32 53 65 41 35 60 66 68 55 109 85 37 80 48 57	43 75 85 81 51 30 47 48 81 81 51 40 55 99 71 91 58 43	94 115 140 96 139 140 128 109 89 104 87 85 150	28 49 88 42 83 37 70 52 44 56

Return selections from the low lines produced on the average slightly higher numbers than low selections in the same generations. Individual cases, however, do not seem to show any definite response. The few return selections made from the

FIG. 8. Daily averages of high and low lines in Ba from February 6 to June 7, 1916. The high lines are represented by continuous lines and the low ones by broken lines. The two upper lines show the daily facet averages while the lower ones represent the actual numbers of flies from which those averages were obtained.

high lines were even less definite. Only one gave offspring that could be distinguished from those of the generation in which they hatched (u4f4–19).

The offspring from the crosses between low and high lines are intermediate and show a very slightly wider range than the low lines, but not nearly so wide a range as the high lines. There is no significant difference between the offspring from high males and low females and those from low males and high females. The apparent difference in the extremes is accounted for by the fact that the standard of all the lines changed during the period of hatching. The results of these matings are given in actual daily counts in Tables III. and IV., and are also shown in Figs. 5 and 7. A comparison of Tables III. and IV. with Fig. 8 shows

that the lower values in the later counts are entirely in agreement with the results obtained at this time in the high and low lines.

Such changes in facet numbers of all the lines had already been noticed in the vestigial-winged race and are clearly shown at several points in Fig. 8. The changes would appear more sudden and the agreement between the lines would be closer if it were not for the fact that the change appeared sooner in bottles that just began to hatch than in older bottles.

The Appearance of Heterozygous Females and Full-Eyed Males.

What developed to be the most puzzling phase of the entire set of experiments was the appearance of full-eyed males and heterozygous females in the stock bottles and in the selected lines during the period they were under observation. Eleven such individuals appeared at different times as shown in Table V.

Table V.

Pedigree of Full-Eyed Males and Heterozygous Females Obtained from Bar-Eyed Stock under Observation.

Sex.	Date of Appearance.	Line.	Facets.	Fate.	Grade of Mates.	Offspring. Bottle No.	♂ Bar.	♂ Full.	♀ Bar.	♀ Het.
♂	Nov. 15, 1915	VBa (Stock)	700?	Mated	43	VBax	102			76
♂	Nov. 29, 1915	VBa (Stock)	1200?	Died						
♂	Dec. 17, 1915	VBah15	1200?	Sterile						
♀	Jan. 16, 1916	VBah3f2	371	Mated	109 / 116	VBah3f2–6	4	4	1	4
♂	Feb. 12, 1916	Bau2	1200?	Mated	61 / 67	Bau2x	13			10
♀	Mar. 10, 1916	Bau2f2–3	601	Died						
♂	Mar. 28, 1916	Bau4f2–10	700?	Mated	149	Bau4f3x	27	17[1]	20[1]	18
♀	Apr. 2, 1916	Bau4f2–13	481	Mated	238 / 204	Bau4f2–13x	3	3	2	4
♀	Apr. 28, 1916	Bau4f4–16	621	Mated	187	Bau4f5x	11	13	7	11
♀	June 6, 1916	Bau4f6–4	452	Killed						
♂	June 7, 1916	Baud8	800?	Killed						

[1] The full-eyed males and bar-eyed females in Bau4f3x were undoubtedly due to the appearance of flies from the second generation before the counts were made. The parents were mated April 3 and by an oversight the offspring were permitted to remain in the bottle until April 27.

The VBa stock in March revealed no such specimens, but the Ba stock in June contained several of each. In that case they were probably due to the appearance of a heterozygous female in the previous generation. One of the stock bottles received from Professor Zeleny contained a mixture of bar-eyed, full-eyed and heterozygous individuals. These may have been due to the appearance of a full-eyed male or a heterozygous female at some previous time.

The eyes of the males could not be distinguished from those of normal wild flies. The facet numbers indicated in the table are mere estimates obtained by counting a row of facets to the middle of the eye and using the number obtained as a radius for computing the number on the entire area. The eyes of wild flies treated in the same way gave similar results. The eye of a wild male which could be counted without turning was found to contain 700 facets.

The eyes of the heterozygous females had the characteristic appearance of the eyes of such females obtained from crosses between wild and bar-eyed flies and contained the corresponding facet numbers. The ordinary bar eye has the shape of a crescent with a notch near the middle of the concave side almost separating the two ends of the crescent. One end is usually considerably larger than the other and frequently contains the irregular facets mentioned before. The eye of the heterozygous female has the shape of the bar eye, but is much larger and does not contain the irregular facets. As shown in the table, the heterozygous females from the long-winged race had considerably larger eyes than those from the vestigial-winged race. This is explained by the fact that the average facet number in the bar eye of the long-winged race is much larger than that of the vestigial-winged race.

Wherever possible the full-eyed males and heterozygous females that appeared were mated, but, with the exception of the first case, no attempt was made to get a large number of offspring or to count the facets in the bar-eyed and heterozygous offspring. Two of the individuals died soon after hatching and the last two could not be mated because the experiments had to be brought to a close. Of those mated one proved to be

sterile and the other six produced offspring. In each case the specimens were mated with bar-eyed flies and with one exception the full-eyed males produced nothing but bar-eyed males and heterozygous females and the heterozygous females produced bar-eyed and full-eyed males and bar-eyed and heterozygous females. The one exception occurred in case of the male which appeared on March 28 and was mated to a bar-eyed female on April 3. The bottle was set aside and overlooked until April 27 when it was found to contain all four classes of flies as shown in the table. In that case the full-eyed males and bar-eyed females are undoubtedly due to the appearance of flies of the second generation before the count was made. Since the first generation consisted of bar-eyed males and heterozygous females the second generation would be expected to contain a mixture of all four classes.

The facets in the eyes of all the offspring obtained from the male appearing on November 15 were counted and the results are given under VBax in Table I. and Fig. 2. A large number of pairs from these offspring was mated, but all proved to be sterile. A mass mating made toward the end of the hatching period, however, produced 14 bar-eyed males with an average facet number of 114, 7 full-eyed males, 12 bar-eyed females with an average facet number of 91 and 5 heterozygous females. These were bred in a stock bottle for several generations to see if one or the other class would become dominant, but no obvious change in the ratios appeared.

Discussion and Conclusions.

The variability of the stock at the beginning of the selections made it appear probable that the facet number is affected by environmental conditions. For that reason the records during the experiments were kept more carefully than would otherwise have been the case. Counts were made and recorded separately at least every twelve hours. Each line or branch of a line was recorded separately, and in cases where parents were transferred and offspring obtained from both bottles a separate record was kept of each. Observations were also made on a possible correlation between body size and facet number.

It was impossible to find any noticeable effect of moisture, consistency, or age of food on the facet number. No significant difference was found in facet number between the first flies hatching from a bottle and the last ones except in cases where the food gradually dried up and the last flies were minute, *i. e.*, one half the size of normal flies or less. Such minute flies showed a tendency toward lower facet numbers. Accurate size measurements and facet counts on a large number of flies may possibly reveal a slight correlation between size of body and number of facets, but mere observation failed to detect it. It is also possible that a slight correlation exists but is concealed by other factors that have more influence.

No relation could be discovered between the age of the parent and the facet number of the offspring. In case of such a relation the latest offspring from two parents would be different from the first. No such difference could be detected.

Likewise it was impossible to find any definite correlation between the length of the developmental period of the larva and the facet number of the adult. No special experiments were undertaken with material in which the exact developmental period was ascertained, but, since in productive bottles the period of hatching is much longer than the period during which the eggs are laid, the first larvæ must as a rule have a shorter developmental period than the last, and any difference due to the length of the developmental period should become evident.

A glance at Fig. 8 shows that beyond the daily fluctuations due to the fact that the number of flies examined was too small to be representative, there are larger, parallel fluctuations of the two lines. Since the food during the Ba selections was fairly constant in character it can not be regarded as the cause of these fluctuations. The temperature of the room in which the flies were kept varied between rather wide limits. Unfortunately no thermograph was available for the room at that time. There is no correlation between the outside temperature and the facet number; but the variations in the room temperature were independent of those in the outside temperature. Since experiments to determine the effect of temperature on the facet number were undertaken by E. W. Seyster of this laboratory during the

latter part of this work and on account of the lack of time no special effort was made to determine the exact relation between temperature and facet number. The fact that the change usually occurred one to three days sooner in bottles that just began to hatch than in old ones can be explained by the assumption that the effect was produced at an early stage in the developmental period. Since the first flies hatching from a bottle must have a shorter developmental period than the later ones they would be the first to show the effect.

It does not seem possible, however, that temperature is the sole cause of somatic variations. In the VBa selections it was observed that the two lines throughout averaged higher than the stock. In spite of that fact an examination of the extremes shows that most of the high flies were eliminated from the low lines. The rank of the average flies must, then, have been raised. The same can be said of the low lines in Ba. Here the elimination of high flies is much more pronounced and still the mean remains very near that of the stock. It is possible that the crowded condition of the larvæ in the stock bottles reduced the facet number, but the real cause may be something very different.

The results of the selections in VBa indicate that there is in this race only a single hereditary factor involved in the modification of the facet number. In spite of the fact that the mean of the stock is much lower than that of either line we must assume that under the same conditions it would lie somewhere between them, in other words, if the lines could have been reared under the conditions of the stock, the low lines would have gone slightly downward due to the elimination of high numbers and the high lines would have gone slightly upward due to the elimination of low numbers. Practically pure lines were established in the first generation. This, however, may be merely apparent, the lack of further effect of selection being due to the interference of somatic factors.

The male with 34 facets, giving rise to the brood 16f1–6, must be regarded as a mutant. This conclusion is based on the great difference between this male and its brothers in 16, the definite relation between the male parent and the female offspring, and the extremely low grade of the resulting females.

In the Ba selections the results are very different and not so easily explained. Here also there must have been a downward shift as well as an upward shift. Disregarding the seventh generation for reasons already given, the following differences between the high and low lines are obtained: f1, 22; f2, 40; f3, 61; f4, 65; f5, 79; f6, 95. There is a gradual increase which becomes somewhat smaller as selection proceeds. If it were not for the interfering fluctuations the author's results would be much like those obtained by Zeleny and Mattoon. Even three additional generations of selection failed to produce pure lines.

In many respects the results obtained in these experiments resemble those obtained by MacDowell in the selection for extra bristles. The average of all the lines is raised or lowered but one can not predict within rather wide limits just what the offspring of any two parents are going to be like. This is clearly shown in Table II. and Fig. 6 in case of the matings d1f1–2 and d1f1–3. These matings were made with the same male but with females of 91 and 56 facets respectively. The average for the offspring from the higher female is considerably lower than that for the offspring of the lower female. In this case the unexpected results can not be explained on the basis that only a part of the character was observed. It is true that the counts were made only on one eye, but the difference between the two eyes falls within definite limits and is very small compared with the differences between the eyes of two flies. A comparable case would be the possibility of less than one extra bristle outside of the observed rows in the experiments of MacDowell. The large, parallel fluctuations of all the lines seem to indicate that there are environmental factors capable of almost doubling or cutting in half the facet numbers of flies of the same germinal constitution. The unexpected results must be regarded as being due to the fact that the hereditary factors are only a part of the total factor group controlling the facet number. When the environmental factors have been studied it may be possible to control them in such a way as to obtain uniform results. In that case more rigid selection will be possible.

The crosses made between the high and low lines show no evidence of sex-linkage. The offspring from both sets of matings

are very nearly alike and there is no essential difference in the ratio of males and females. This agrees with the results obtained by MacDowell in his work on extra bristles. In the present experiments sex-linkage might have been expected from the fact that the ratio between male and female averages did not remain constant from generation to generation. The factors for converting the female means to the male standard in the seven generations of upward selection are successively 1.91, 1.73, 1.53, 1.40, 1.36, 1.60, 1.57. Those for the downward selections are 1.57, 1.86, 1.43, 1.79, 1.51, 1.46 and 1.40. But it is impossible to relate the differences in the offspring to differences in the parents.

The appearance of full-eyed males and heterozygous females in the stock and selected lines may be explained in two ways. It may have been due to the carelessness of the author in preparing the food, feeding and transferring the flies, or it may have been due to reverse mutation in the race.

The precautions taken by the author were not such as to exclude absolutely the possibility of the entrance of an egg here and there. But in spite of that fact the evidence is almost irrefutably against the theory of contamination. Perhaps it is well to state here in detail the author's methods. The precautions in regard to the preparation of food have already been given. It is sufficient to add that no larvæ ever appeared in the food jar. In all of these experiments the food was handled by means of an all-metal scalpel. This was used chiefly because it could be easily cleaned and could be kept absolutely clean. During the first two generations of VBa selections the author depended upon the fact that *Drosophila* does not as a rule lay eggs on a clean, dry, metal surface and merely kept the scalpel clean but did not heat it before using. In all succeeding work it was heated in an alcohol flame just before being used. All vials and bottles that had been used were boiled and rinsed in tap water and were then kept inverted on the table until they were again used. In the VBa selections no filter paper was used with the food. In the Ba selections the clean filter paper was kept in a table drawer and was removed only at the time of using. Fresh cotton for the plugs was also kept in table drawers.

If old plugs were used over again they were either sterilized and kept in closed fruit jars or they were kept in closed jars for at least a week before using. Only plugs that had not been contaminated with food were used again.

But the evidence against the theory of contamination has little to do with the precautions taken in handling the material. In the first place all flies that appeared in the vestigial-winged lines had vestigial wings and all flies that appeared in the long-winged lines had long wings. The chief reason for choosing the vestigial-winged race for the selections was the fact that the second recessive could be used as a check in case of contamination. In all of the experiments no long-winged fly appeared in the vestigial-winged lines and no vestigial-winged form appeared in the long-winged lines. If the full-eyed and heterozygous flies had been due to contamination then the other characters should also have appeared, especially since for a long time the breeding vials of the two races were intermingled and treated as one lot. In the second place the author was handling no full-eyed, vestigial-winged flies at the time the first male appeared. It is true that Professor Zeleny had his stock of such flies in the same room at the time, but they were kept on a table about twenty feet from the one used for this work and the chances that a vestigial-winged fly will travel that distance are not very great. Finally, if the flies were due to contamination, full-eyed females as well as full-eyed males should have appeared; indeed, full-eyed females should have been more frequent than heterozygous females, but all females were heterozygous.

It is interesting to note that in all cases except one where full-eyed or heterozygous flies appeared in the selected lines it was in the high lines. The one exception was the heterozygous female in h3f2, a reverse selection from a low line.

More significant, however, is the fact that the females were always heterozygous. That means that if the change took place after fertilization only one chromosome in case of the females was affected. The males, of course, have only one chromosome bearing the factor. If the change occurred before fertilization, then it is probable that it appeared in the female germ cells. Had it taken place also in the male germ cells then

heterozygous females should have appeared more frequently than full-eyed males and full-eyed females might in rare instances have appeared. If the change occurred indiscriminately in male or female then the proportion of heterozygous females to full-eyed males should be 3 to 1, but the ratio obtained is 5 to 6. The numbers obtained, however, are not large enough to be conclusive.

The fact of reverse mutation is very difficult to explain. It is hard to see how the germplasm can lose a factor and still potentially retain it and have it reappear later. So far as the present data go it is possible to explain the case under consideration in two different ways.

If the normal wild fly carries a limiting factor with respect to the facet number then it is possible by partial non-disjunction for the factor to pass from one chromosome of a pair to the other, giving one chromosome without a limiting factor and the other with a double limiting factor. The bar-eyed race of *Drosophila* may be derived from such a chromosome with two limiting factors or factor groups, the mate of the chromosome having been lost in the maturation of the egg. If then in the bar-eyed race a second non-disjunction again separates the two factors the result should be one chromosome with triple factors and one with a single factor, the latter giving rise to a full-eyed male or a heterozygous female. If the former passes into the egg it should give rise to a further reduction in the facet number, but it is possible that a fly with such a chromosome does not live. It is possible also that the male with 34 facets contained a chromosome with a triple reducing factor.

A simpler explanation is that of a reversible chemical change between two compounds one of which is more stable than the other. If the compound that forms the basis for the bar eye is the less stable then reversions are to be expected under certain conditions. But the fact that the change takes place only in the female is a strong argument in favor of the theory of partial non-disjunction.

Summary.

Selection was carried on in the vestigial-winged, bar-eyed stock for three generations and in the long-winged, bar-eyed

stock for seven generations. The facets in the eyes of 9,000 flies were counted.

Selection in the vestigial-winged stock had to be discontinued on account of sterility and low production in the single lines.

The sterility and low production were not due to inbreeding. Sterility was not due to inability on the part of the females to lay eggs, nor does it appear to have been the final stage in the reduction of the number of offspring.

Selection in the vestigial-winged stock was effective for one generation but failed to produce further effects in the second and third generations. Return selections from the low lines were not effective.

A mutation appeared in a male in line 16. It was a reducing factor and was sex-linked. The complete sterility of the offspring of this male prevented further study of the character.

Selection in the long-winged stock was effective for six generations. In the downward selected lines most of the high flies were eliminated in the first three generations and no further effect of selection was noted. Return selections from the sixth generation, however, were still effective. The mean of the upward selected lines continued to increase at approximately uniform rate for six generations. The seventh generation must be disregarded because it was not an upward selection. The range in the facet number was not increased by selection. The results of these experiments indicate that the hereditary differences in this race of *Drosophila* are due to a large number of small factors.

Crosses made in the sixth generation between low and high lines indicate that the hereditary factors are not sex-linked.

In both the long-winged and the vestigial-winged lines the mean facet number was highly variable. This variability appeared to be due chiefly to changes in the temperature of the room, but may have been due also to other causes. The mean facet number of flies reared in vials as single lines was slightly higher than that of stock flies reared in bottles.

The mean facet number of the vestigial-winged flies was somewhat lower than that of the long-winged flies.

Six full-eyed males and five heterozygous females appeared in the stock bottles and the selected lines during these experiments. They must be regarded as reverse mutations.

The reverse mutation can be explained on the theory of a reversible chemical reaction between two compounds one of which is more stable than the other. The present facts, however, favor an explanation on the basis of partial non-disjunction.

LITERATURE CITED.

Castle, W. E., Carpenter, T. W., Clark, A. H., Mast, S. O., and Barrows, W. M.

'16 The Effect of Inbreeding, Cross Breeding and Variability of *Drosophila*. Proc. Amer. Acad. Arts. Sci., 41: 731–786.

Hyde, Roscoe R.

'14 Fertility and Sterility in *Drosophila ampelophila*. I., Sterility in *Drosophila* with Special Reference to a Defect in the Female and its Behavior in Heredity. II., Fertility in *Drosophila* and its Behavior in Heredity. Jour. Exp. Zoöl., 17: 141–212.

MacDowell, E. Carlton.

'15 Bristle Inheritance in *Drosophila*. I., Extra Bristles. Jour. Exp. Zoöl., 19: 61–98.

'16 The Influence of Selection on the Number of Extra Bristles in *Drosophila*. Science, N. S., 43: 177.

Pearl, R.

'12 The Mode of Inheritance of Fecundity in the Domestic Fowl. Jour. Exp. Zoöl., 13: 153–268. (Gives list of other papers.)

Zeleny, Charles, and Mattoon, E. W.

'15 The Effect of Selection upon the "Bar Eye" Mutant of *Drosophila*. Jour. Exp. Zoöl., 19: 515–529.

MITOSIS AND AMITOSIS.

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It was once supposed that all forms of nuclear division were of the type which is now called amitosis in which the nucleus undergoes simple constriction. After the discovery of the complicated method of nuclear division now known as mitosis and the recognition of its very general occurrence in plants and animals, the doubt was expressed whether amitosis ever occurs as a normal process; its occurrence in pathological and degenerating cells is undoubted. However, the number of cases in which nuclear constriction is known to occur regularly in certain tissue cells is too great to warrant the belief that it is always a pathological phenomenon.

The only question of present importance in connection with this form of nuclear division is as to its bearing on the chromosome theory of heredity. If amitotic division of the nucleus, followed by division of the cell body, ever occurs in the germ cell cycle or in cleavage cells undergoing normal differentiation it would go far to disprove the "individuality" or persistent identity of chromosomes and consequently the chromosome theory of inheritance, since it is scarcely possible that individual chromosomes can be equally divided and their halves accurately distributed to daughter cells by the process of amitosis. The majority of cytologists at the present day concur in the opinion that amitosis does not normally occur in embryonic or germ cells and that when it does occur in such cells the ensuing development is abnormal. On the other hand some investigators maintain that amitosis occurs regularly and predominantly in the genesis of the germ cells and also in the blastomeres and embryonic cells of several species of animals and plants and for this reason among others they reject the chromosome theory. Child in particular has maintained this position in a series of papers (1907–1911) dealing primarily with the cestode *Monezia*,

but secondarily with several other animal forms ranging all the way from cœlenterates to birds.

Among other recent writers who have maintained a similar view are Hargitt (1904, 1911) on amitosis in the cleavage of the eggs of cœlenterates, Patterson (1908) in blastoderm cells of the pigeon, Glaser (1908) in embryonic cells of *Fasciolaria*, Jordan (1908) in spermatogonial divisions of *Aplopus*, Nathansohn (1900) in *Spirogyra*, Wasielewski (1902, 1903) in the root tips of *Vicia*, Gurwitsch (1905) in the blastomeres of *Triton*, Maximow (1908) in embryonic tissues of the rabbit, Jörgensen (1908) in the oögenesis of *Nephelis*, Moroff (1909) in the eggs of copepods, Knoche (1910) in the insect ovary, Nowikoff (1910) in bone and sinew cells of the young mouse, and Foot and Strobell (1911) in the ovary of *Protenor*—all of whom hold that amitosis may occur as a normal process in germinal and embryonic cells. Several of the authors named as well as R. Hertwig (1898), Lang (1901), Calkins (1901), Herbst (1909), Godlewski (1909) and Konopacki (1911) hold that there is no principal distinction between mitosis and amitosis and that they may both occur without interfering with normal processes of differentiation.

On the other hand, this view is contested by Boveri (1907) and Strasburger (1908) on general grounds and is not confirmed by the experiments of Häcker (1900) and Schiller (1909) on *Cyclops* eggs subjected to ether, nor by the experiments of Němec (1903) who repeated the work of Wasielewski on the root tips of *Vicia* subjected to chloral hydrate and reached the conclusion that the supposed amitoses are really modified mitoses. Richards (1909, 1911) and Harmon (1913) carefully and laboriously repeated Child's work on cestodes and found no evidence of amitosis in germ cells or cleavage cells, while on the other hand there was abundant evidence of mitosis. Child (1911) has reëxamined the question and reaffirms in the main his former opinion, and this has been confirmed by Young (1913), whose general conclusions, however, are so reactionary and even revolutionary that they cannot be accepted without further confirmation.

Boveri (1907) has said that if Child would establish his contention he must prove (1) that the binucleate condition which

he finds is actually due to a division, (2) that a portion of the cytoplasm is cut off around each of these nuclei, (3) that the cells arising in this manner again divide mitotically and have the normal number of chromosomes; in his opinion not one of these proofs has been furnished. The binucleate or multinucleate condition, he adds, may be due to an incomplete fusion of chromosomal vesicles after mitosis, as Rubaschkin (1905) has shown to be true for the blastomeres of *Triton*. Boveri concludes, therefore, that the individuality theory has not been shaken by this work on amitosis. There is no doubt that constricted or lobulated nuclei are sometimes found in germ cells or embryonic cells; the critical question is whether these are stages in the amitotic division of the nucleus and if so whether the cell bodies divide and are capable of normal development. It is evident from a large number of observations on a great variety of objects that constricted nuclei are not in themselves sufficient evidence that amitosis is occurring, for many such nuclei of living cells have been observed to lose the constriction and later to divide by mitosis. And even if such constricted nuclei actually divide, the chromosomal constitution of the cell could remain normal provided the cell body did not divide following the amitotic division of the nucleus.

As a matter of fact, amitotic division of the nucleus is rarely followed by division of the cell body. Macklin (1916) has recently studied amitosis in living tissue cells of the embryo chick and, although he was able to follow the process of nuclear division in successive stages in one and the same cell, in no case was such amitotic division of the nucleus followed by division of the cell body. Such amitotic division was sometimes followed by mitosis and in such cases the two or more nuclear vesicles in a cell gave rise to chromosomes which formed a single equatorial plate. Macklin concludes, therefore, that "there is nothing in nuclear amitosis opposed to the chromosome hypothesis"—with which opinion I entirely agree.

Unfortunately it is rarely possible to study all stages of nuclear and cell division in normal living cells, not only because of the indistinctness with which one sees what is going on inside the cell, but also because of the difficulty of keeping cells alive and

normal for a sufficient length of time under conditions of observation; furthermore, failure to observe amitosis in one hundred such cells would still leave it possible that it might occur in a second hundred. There is, however, a method by which the occurrence or non-occurrence of amitosis can be determined with great certainty and this is in the study of identical cell divisions in hundreds and thousands of individuals. This is possible only in the two maturation divisions which are so peculiar that they can always be distinguished from each other and from all other divisions, and in those early cleavages where the lineage of every cell is known and its method of division can be observed in thousands of different cases. If in every such dividing cell one sees mitosis only, it can be concluded that this is the invariable method of division in these cells. It is a significant fact, which Child himself admits, that in no such case have the nucleus and cell ever been seen to divide by amitosis, whereas in hundreds and thousands of cases they have been seen to divide by mitosis.

Nevertheless, in these very cells one frequently observes lobulated, constricted or bipartite nuclei which might be regarded as stages in amitosis were it not for the fact that the study of the lineage of these cells shows that they invariably divide by mitosis and that the peculiar shapes of the nuclei referred to are due to modifications of normal mitosis. Such constricted or bipartite nuclei occurring in cells the lineage of which was unknown would naturally be mistaken for cases of amitosis and it is very significant that the reports of amitosis in embryonic cells have been invariably in cells of unknown lineage.

This paper is based on a study of modified mitosis in the maturation and cleavage of the eggs of the marine gasteropod, *Crepidula plana*. A variety of nuclear forms are figured and described which resemble more or less closely stages of amitosis and yet it is perfectly certain that these nuclei divide only by mitosis, and the manner of origin of these pseudo-amitotic forms is plainly due to modifications of regular mitotic processes. These modifications were produced by subjecting dividing cells to abnormally high temperatures or to sea-water of abnormal densities, but other similar modifications are produced by many other abnormal conditions such as pressure, centrifugal force,

carbonic acid and various other chemical substances (Conklin, 1912). These different types of modified mitoses may be classified under the following heads: (1) Scattering of chromosomes and their failure to unite into a single nuclear vesicle, (2) amitotic or mitotic division of the nucleus without division of the cell body and the subsequent division of such binuclear or polynuclear cells, (3) separation of chromatin and achromatin and formation of cytasters, (4) persistence of nuclear membrane and formation of chromatic connections between daughter nuclei.

I. Scattering of Chromosomes and Their Failure to Unite Into a Single Nuclear Vesicle.

In the late anaphase of normal mitoses the chromosomes of the daughter plate stick together so that when the individual chromosomes begin to take in achromatic substance and to swell up into chromosomal vesicles the whole plate is converted into a mulberry-like mass which later becomes a single nuclear vesicle either by the fusion of the separate chromosomal vesicles or by their closer approximation. There is a growing body of evidence that in certain cases at least these closely appressed chromosomal vesicles do not completely fuse with one another but preserve their individuality (Bonnevie, 1908, for *Ascaris* and *Allium*; Vejdovsky, 1912, for *Ascaris* and *Decticus*; Wenrich, 1916, for *Phrynotettix*; Richards, 1917, for *Fundulus*). In other cases, when it is not possible to recognize a distinct vesicle for each and every chromosome, maternal and paternal chromosomes may form more or less distinct vesicles (Häcker, 1895, for *Cyclops*; Conklin, 1901, 1902, for *Crepidula*).

In certain abnormal conditions, and especially by means of temperatures higher than normal and by hypertonic sea-water, the division and separation of daughter chromosomes may be delayed or stopped and the chromosomes scattered along the length of the spindle (Figs. 9, 10, 29). After the chromosomes have reached the poles of the spindle, they may be separated from one another and remain scattered more or less widely in the cell. If the temperature is not too high (34°–35° C.) each separate chromosome will then swell up to form a separate vesicle, or if two or more chromosomes are in close contact they may form a single vesicle of larger size (Figs. 7, 9–12).

If the temperature is a little higher (37° C.) the chromosomes may stick together in an irregular mass and be drawn to the surface of the cell, probably by the complete contraction of the fibers which anchor the spindle to the peripheral layer of the protoplasm (Figs. 13, 14, 17). In the latter instance the chromosomes do not swell up and become vesicles, but remain permanently small and densely chromatic. Owing to some change, probably in the peripheral layer of each chromosome, caused by the high temperature, the chromosomes are unable to take up achromatin. Such chromosomes and the cells containing them never recover and never go further in development, although the cells do not immediately undergo degeneration and to all appearances remain alive for twenty-four hours or longer.

Of a piece with this scattering of chromosomes is the failure of all the chromosomal vesicles to unite into a single vesicle. All degrees of fusion of chromosomal vesicles may be found from those which remain wholly separate to those which are united into a single spherical vesicle. Other things being equal, the size of a nuclear vesicle varies according to the number of chromosomal vesicles which enter into it. In this way arise nuclear forms which have been called "fragmented nuclei," "multinuclear cells," "bipartite," "lobulated" and "elongated" nuclei (Figs. 1–12, 19–28), although it is evident in the case of *Crepidula* that these nuclear forms have not arisen by constriction or fragmentation of an originally single nucleus.

These partial nuclei formed by incomplete fusion of the chromosomal vesicles were first called "karyomeres" by Fol. That they are partial and not entire nuclei is shown by the fact first established by Boveri that in the next following mitosis each karyomere gives rise only to the same number of chromosomes as entered into it and not to the full number of chromosomes characteristic of the species; this fact I have repeatedly confirmed in my studies on *Crepidula*. And that such karyomeres are due to the failure of chromosomes to unite into one vesicle and not to the amitotic division of a single vesicle is shown by the following considerations:

1. They are most numerous in the telophase of division (Figs. 7–12) where the chromosomes are sometimes widely scattered

and where every chromosome may give rise to a separate chromosomal vesicle (Fig. 7). As the cell passes into the resting phase, these vesicles fuse together more or less completely, giving rise to vesicles of varying sizes. Other things being equal, the later the stage in the resting period the smaller the number of separate vesicles. If the separate vesicles were formed by division of an originally single vesicle, exactly the reverse would be the case.

2. The presence of centrospheres and spindle remnants in many cases shows conclusively that division has taken place by mitosis, and the position of these structures indicates the location of the mitotic figure (Figs. 8–12 *et seq.*).

3. The elongation of daughter nuclei in the position of the plate of daughter chromosomes of normal division figures indicates that such elongated nuclei are formed by the partial fusion of the chromosomes of the daughter plate (Figs. 4–6).

These considerations make it absolutely certain that these peculiar nuclear forms are due to a partial or incomplete union of chromosomes into a single nuclear vesicle in the final phase of mitosis; they represent modified mitosis and not amitosis. And it is practically certain that many of the cases of so-called amitosis described by several of the authors mentioned above are of this same character.

It is not easy to determine exactly the mechanism by which these modifications have been produced. As already mentioned they may be caused by abnormally high temperature (Figs. 1–12) or by hypertonic sea-water (Figs. 19–29). It is well known that when the daughter chromosomes approach the poles of the spindle they are normally closely crowded together and it seems probable that this is due to linin connections within the spindle or between the chromosomes. In the development of the daughter nucleus each chromosome absorbs achromatic material from the cytoplasm and becomes a vesicle with chromatic walls. The material thus absorbed is probably chiefly water though it doubtless contains dialyzable proteins and other substances which may be assimilated into the chromatin and linin of the nucleus. It is probable that there is a real membrane surrounding each chromosome (Conklin, 1902, 1912) and that the

absorption of surrounding substances by the chromosome takes place through this membrane by a process of dialysis. The nuclear vesicle is most nearly spherical in form when it is largest and when its contents are most fluid in character irregular or lobulated nuclei are usually smaller and the nuclear contents more dense (Figs. 1–6). Therefore the modifications of mitosis, which prevent the union of chromosomes into a single vesicle, act by modifying the walls of these vesicles so that they do not readily unite and so that they do not readily absorb fluid from the cytoplasm.

II. Amitotic or Mitotic Division of Nuclei Without Division of Cell Body and Subsequent Division of Such Binuclear or Polynuclear Cells.

In the cleavage of *Crepidula* a binucleate or multinucleate cell is invariably due, so far as I have observed, to a failure of chromosomal vesicles to unite into a single vesicle. In other cases, however, it is plain that elongated, constricted and bipartite nuclei have resulted from the constriction of a single nuclear vesicle. In addition to numerous cases which have been described by other investigators, I have myself studied such cases in the egg-follicle cells of *Gryllus* (Conklin, 1903), the liver cells of *Porcellio* (Conklin, 1897), as well as in some muscle cells and connective tissue cells. In none of these cases, however, is division of the nucleus followed by division of the cell body. Although every follicle cell of *Gryllus* and every liver cell of *Porcellio* shows the nucleus in some stage of amitosis, and although many of these cells contain two entirely separate nuclei, in no single instance have I ever seen a division of the cell body separating these nuclear halves.

Since these cases of amitosis occur in differentiated tissue cells it may be assumed that the nuclei are not active in the further differentiation of these cells; on the other hand their metabolic activity is great and the nuclei are undoubtedly concerned in this activity. It has been assumed that the division or lobulation of such nuclei favors metabolic activity by increasing the surface of the nuclei and bringing them into closer relation to all of the cytoplasm of the cell, and this is probably true. Espe-

cially in the case of elongated muscle cells the division of the nucleus and the distribution of the daughter nuclei along the length of the fiber must facilitate interaction between nucleus and cytoplasm, and the same is true, though perhaps to a smaller extent, in gland cells and egg-follicle cells.

Dahlgren and Kepner (1908) hold that the very numerous amitoses in the striated muscle cells of the embryo fish, *Catostomus*, may be followed in some instances by the division of the muscle cell. But since the plane of nuclear division is always transverse to the fiber, while the plane of cell division is always longitudinal, it could not be affirmed that the cell divisions in this case correspond to the nuclear divisions. But even if amitoses may be followed by division of the cell body in these cases, it must not be forgotten that all these cells are fully differentiated and according to the chromosome theory the nucleus has already performed its differentiating functions while its further function in the fully differentiated cell is probably purely trophic.

Many observations and experiments demonstrate that the nucleus is concerned in the two functions (1) of differentiation or regulation and (2) of metabolism; the work of Gruber (1886), R. Hertwig (1898), Heidenhain (1894), Henneguy (1896), Conklin (1902) *et al.* indicates that the chromosomes or basichromatin are particularly concerned with the former, the oxychromatin or achromatin with the latter. It is a significant fact that chromosomes divide only by mitosis and Boveri (1908) has shown that a complete set of chromosomes is necessary to normal differentiation. On the other hand oxychromatin and achromatin divide only by amitosis even in cases of mitotic division of the chromosomes. The significance of these facts seems to have been missed not only by those who maintain the equivalence of mitosis and amitosis, but also by Weismann and his followers who assumed that in embryonic differentiation there is a differential division of chromosomes and a "disintegration of the germ plasm" with segregation of particular factors in particular cells. For if individual chromosomes differ in hereditary potencies, every division by amitosis must be a differential one, while on the other hand every typical mitotic

division is non-differential so far as the chromosomes are concerned.

Since it is usually impossible to see outlines of individual chromosomal vesicles in the resting nucleus, it is not possible to determine whether the constrictions and lobulations of amitosis merely separate whole vesicles from one another. If they do the number of chromosomes arising from each of these vesicles in subsequent mitosis should be the same as in the case of karyomeres formed by the failure of vesicles to unite in the resting stage.

It is probable from the work of Boveri (1907) and of Macklin (1916) that when amitotic division of the nucleus is followed by mitosis, each nuclear vesicle gives rise to a fraction only of the normal number of chromosomes and that all the nuclear vesicles in a cell taken together give rise to no more than the normal number. Furthermore, the work of Boveri demonstrates that there is no return to the normal number of chromosomes when once a cell contains an abnormal number. Each nuclear vesicle produced by amitotic division is therefore a karyomere, in every way comparable to those produced by incomplete fusion of chromosomal vesicles; it is a fragment of a nucleus and not an entire nucleus, and this is equally true whether all the karyomeres lie within a single cell, as is usually the case, or whether in some rare instances they may be distributed to separate cells. In *Crepidula* it matters not how many karyomeres there are in a cell, if there are two and only two centrosomes all the chromosomes come together into a single plate and there is a normal division and distribution of each of these chromosomes to the daughter cells.

Therefore in considering the significance of amitosis it is of the utmost importance to know whether the constriction of the nucleus is followed by a division of the cell body; if it is not, amitosis is not a permanent nuclear division at all but merely a temporary separation of karyomeres which come together again into a unit structure at the next mitosis. It is a significant fact that in most instances amitotic division of the nucleus is not followed by division of the cell body.

In this connection it is worth while to compare with the

conditions just described those which obtain when mitosis is not followed by division of the cell-body. In a former paper (Conklin, 1912) I have described such cases at some length and need not here go into details. In brief, if the daughter nuclei and centrosomes lie so far apart in the undivided cell body that they do not interfere, in subsequent mitoses every one of these mitoses may be entirely normal and development may be typical except that no differentiation ever appears between the halves of the undivided cell. On the other hand, if the daughter centrosomes lie near together in the undivided cell body so that they interfere we get tripolar or tetrapolar figures with irregular distribution of chromosomes and usually with the formation of several karyomeres of varying sizes depending upon the number of chromosomes entering into them. Such multipolar mitoses in *Crepidula* are rarely followed by division of the cell body so that at every succeeding mitotic period the number of centrosomes and chromosomes in this undivided cell body are approximately doubled (Figs. 26–29). Of course such cells with abnormal numbers of chromosomes and centrosomes never develop normally. Normal differentiation depends upon the regular distribution into separate cells of daughter centrosomes and chromosomes as well as of different cytoplasmic substances.

III. Separation of Chromatin and Achromatin and Formation of Cytasters.

The behavior of the chromatic and achromatic parts of the nucleus in hypertonic and in hypotonic media throws a certain amount of light on the constitution of the normal nucleus and on the behavior of these nuclear constituents during normal mitosis. When resting nuclei are subjected to hypertonic solutions (*e. g.*, 2–4 per cent. NaCl in sea-water) the chromatic portion of the nucleus contracts into a small dense mass leaving the achromatic portion as large as ever (Figs. 32, 43, 48). It looks as if the chromatin had undergone complete "plasmolysis" whereas the volume of the achromatin had not been affected at all. The membrane or boundary of this achromatin remains full and unshrunken, which would presumably not be the case if this outline represented a real plasma membrane. The

shrinkage of the chromatin on the other hand probably indicates that it is surrounded by a plasma membrane, or more likely that each chromosome is so surrounded.

When resting nuclei are subjected to hypotonic solutions the entire nucleus becomes slightly swollen and less deeply chromatic, which indicates that the chromatic parts of the nuclei take up water, probably through the chromatic nuclear membrane or the chromosomal membranes.

The achromatic membrane, or rather boundary, is regularly spherical in resting stages but during mitosis it disappears or else becomes so indefinite and irregular in outline that it is difficult to recognize. However the achromatic substance of the nucleus together with some of the denser portion of the cytoplasm constitutes the amphiaster with its nuclear spindle and astral radiations. In hypertonic solutions the amphiaster is sharply set off from the surrounding cytoplasm (Figs. 35–36), due as I believe in the main to the condensation of its substance and the elimination from it of the more fluid cytoplasm. In this process of condensation the astral radiations are largely drawn into the central part of the figure but portions of these radiations may become isolated from the amphiaster and thus form independent condensation centers. These have a radiating structure and are typical cytasters, but unlike those described by Wilson they do not in *Crepidula* divide nor form the poles of true mitotic figures. My observations on the origin and nature of these cytasters (Conklin, 1912) entirely agree with those of Konopacki (1911) and in the main with the observations of Mead (1898) and Morgan (1899).

Cytasters appear best developed during periods of mitosis when the achromatin is distributed in the astral radiations (Figs. 33–35) but they are also abundant in eggs after the maturation divisions and before the first cleavage (Figs. 31, 32) and in such cases one can frequently see that they lie along the radiations of the maturation aster (Fig. 31).

During prolonged resting periods, especially when the eggs are in strong salt solutions, cytasters are replaced by faintly staining vesicles (Figs. 37–42) which appear to contain achromatic nuclear material. These vesicles are surrounded by a delicate achromatic

membrane and they resemble R. Hertwig's "nuclei without chromatin." They are found chiefly in the position of the previous spindle remnants and along the lines of astral radiations. Usually the largest of these achromatic vesicles are in close proximity to the dense mass of chromatin, which in these cases does not become vesiculated. In some instances there is a single elongated achromatic vesicle in each daughter cell which occupies the position of the interzonal fibers of the spindle (Figs. 43–48) and which may inclose the dense mass of chromosomes at the spindle ends. Such conditions give the appearance of an amitotic division of the nuclear vesicle, but the presence of centrosomes, mid-bodies and in some instances of spindle fibers and astral radiations as well as of chromosomal plates (Figs. 46, 47) clearly shows that these divisions are true mitoses in which the chromosomes have been prevented from absorbing achromatin while the latter has formed a definite boundary or membrane separating it from the cytoplasm.

Just as the size of a central aster is reduced by the presence of numerous cytasters or parasitic asters which surround it, so the size of the chromatic nuclear vesicle is inversely proportional to the volume of the achromatic vesicles in the cell. It seems practically certain that the chromosomal vesicles and consequently the entire chromatic portion of the nucleus grow by the absorption of this achromatic substance. When nuclei are large they contain much achromatic substance and at the same time there are no cytasters or achromatic vesicles in their vicinity; when they are small and densely chromatic there may be cytasters in the cell during the periods of mitosis, or achromatic vesicles during resting periods. "The cytasters are therefore, in my opinion, isolated portions of archiplasm (achromatin plus spongioplasm) derived in large part from escaped achromatin, which take the aster form during mitosis and the vesicular form during resting periods" (Conklin, 1912, p. 543).

IV. Persistence of Nuclear Membranes and Formation of Chromatic Connections Between Daughter Nuclei.

It has generally been assumed that one of the strongest evidences that amitosis had occurred in any given case was to be

found in the incomplete separation of daughter nuclei or in chromatic connections between them. Thus Gurwitsch (1905) has figured and described the division of a blastomere of a centrifuged *Triton* egg in which two nuclei, connected by a chromatic thread, are dividing by mitosis. The chromatic connection is taken as proof positive that the nuclei had divided by amitosis and Godlewski (1909) in a general review of this subject, after dismissing as doubtful many other cases in which amitosis had been reported as occurring in normal development, falls back upon this case described by Gurwitsch as one of the strongest evidences in favor of the view that amitosis may occur in normally differentiating cells.

But chromatic connections between nuclei are not to be taken as positive evidence that those nuclei have divided by amitosis, for these connections may be the result of incomplete or atypical mitoses. Anything which retards or prevents the separation of daughter chromosomes may lead to the scattering of chromosomes along the spindle or to their elongation into threads and consequently to the formation of chromatic connections between daughter nuclei. Häcker found such connections in etherized eggs of *Cyclops* and such connections are present also in *Crepidula* eggs subjected to high temperatures (Figs. 6, 8), to hypertonic sea-water (Fig. 29), and to hypotonic sea-water (Figs. 49–60). It is especially in the last named experiments that chromatic connections between daughter nuclei are most frequently seen and they merit a detailed description.

The eggs shown in Figs. 49–54 were placed for one hour in sea-water diluted with two volumes of fresh water and were then returned to normal sea-water for four hours before being fixed; those shown in Figs. 55–60 were placed for two hours in sea-water diluted with one volume of fresh water and were then left in normal sea-water for fourteen hours. In all of these cases the centrosomes divided normally and approximately normal spindles were formed but the separation of chromosomes and the formation of daughter nuclei were atypical. In Figs. 9, 29, 49 and 50 the scattering of chromosomes is shown in some of the spindles but more notable than this is the stretching of chromosomes into long threads some of which run from one

pole of the spindle to the other. When chromosomes are merely scattered throughout the cell or along the spindle they usually give rise to chromosomal vesicles wherever they lie, as is shown in Figs. 7, 16, 26, etc., but when in addition they are stretched into elongated threads chromatic connections are left between daughter nuclei.

In diluted sea-water the chromosomes show a tendency to stick together into masses and to stretch out into long threads instead of dividing and moving to the two poles of the spindle. This is probably due to the fact that the linin basis of the chromosomes is modified so that the latter do not preserve their usual shapes and do not separate normally in division. The nuclear membrane also frequently remains chromatic and in such cases may persist throughout mitosis (Figs. 49–53, 55, 59, etc.). Evidently some of the chromatin which usually enters into the formation of the chromosomes is left in these cases at the periphery of the nucleus. Since the nucleus is composed of chromosomal vesicles more or less completely united this result might conceivably be due to the swelling and bursting of some of these vesicles at the nuclear periphery.

When mitosis is halted in the prophase of the third cleavage the centrosomes separate and a spindle is formed in the usual manner but the nuclear membrane persists and the entire nucleus becomes pear-shaped (Fig. 49, cell D), or unequally constricted (Figs. 51, 52), the smaller portion corresponding to the micromeres containing almost all of the deeply staining chromatin in the form of chromatic threads, while the larger portion belonging to the macromeres contains faintly staining threads and granules. These two portions of the constricted nuclei are approximately proportional in size to the nuclei of the normal micromeres and macromeres, although in cell D, Fig. 49, and cells C and D, Fig. 52, the division wall between the micromeres and macromeres has not formed. The fact that these two portions of the constricted nuclei are proportional in size to the cells to which they belong even when the division wall between those cells has not formed is difficult to explain. Generally the size of a nucleus is proportional to the volume of cytoplasm in which it lies (Conklin, 1912) because the chromosomal vesicles

absorb substance from the cytoplasm in their growth, but in this case the entire nucleus has undergone constriction and the cell body has not.

The aggregation of chromatin on the side of the nuclear vesicle on which the spindle lies causes it to collect on the animal pole side of the nucleus in Figs. 49–51 and on the side away from the animal pole in cell D, Fig. 54. In every instance the chromatin collects at that pole of the nucleus which is next to the centrosome or spindle; this is a general phenomenon which has been remarked by R. Hertwig in *Actinospherium*, by Calkins in *Noctiluca*, Conklin in *Crepidula*, etc. In this connection one recalls that in many protozoa the nuclear membrane persists throughout mitosis the spindle being within the nuclear vesicle. The chromosomes divide and separate as in typical mitosis but the nuclear membrane and vesicle constrict as in amitosis. In the ciliata the micronucleus divides by mitosis, the macronucleus by amitosis. In metazoa also the chromosomes alone divide by mitosis, or the splitting of the chromatic thread, while the division of all other nuclear constituents is a mass division.

The peculiar form of nuclear division which is caused by the stretching out of the chromosomes and the persistence of the nuclear membrane superficially resembles amitosis but is really a modified form of mitosis. Nuclei which have divided in this peculiar manner go on dividing by mitosis when they are returned to normal sea-water. Thus cells A and C, Figs. 49 and 50, cells A and B, Figs. 52 and 53, and all the macromeres in Fig. 54 are shown dividing by mitosis. These are typical mitotic figures though the number and arrangement of chromosomes may be atypical. In all of these cases the dividing nucleus of the macromere is connected with the nucleus of the micromere by a chromatic strand and it is particularly noticeable that this strand always runs to the plate of chromosomes in the macromeres and usually to the outer side of this plate. Since the original chromatic connection united the nuclei by their distal poles (away from the centrosome) the fact that when these nuclei divide the connection runs to the proximal pole (toward the centrosome) indicates that the chromatin changes position within the nuclear vesicle, being drawn to the proximal pole of the nucleus (Fig. 54, *D*).

Again the way in which the chromatic connection between nuclei unites with the chromosome plate (Figs. 53, 54) shows that this connection is actually composed of chromosomal substance though it has been so modified that it does not give rise to separate chromosomes nor does it show any tendency to divide or split, as normal chromosomes do, into daughter chromosomes. Although it is not possible to count the number of chromosomes in these plates it is evident that it varies in different cases and that in general it is less than normal, which is what would be expected if the chromatic connections represent a number of spun-out chromosomes. Furthermore the fact that these connections do not swell up to form vesicles as normal chromosomes do, indicates that the chromosomal substance of which it is composed has undergone some significant change.

Still later divisions of the cells connected by these chromatic strands are generally abnormal, as is shown in Figs. 55–60. The eggs shown in these figures were subjected to diluted sea-water during the third cleavage and were then returned to normal sea-water where some of the cells have undergone the fourth, fifth and sixth cleavages. In many cases the chromosomes at each of these cleavages have been stretched out into chromatic connections between daughter nuclei and since all of these persist a complicated network of such connections is present between nuclei of successive generations (Figs. 55, 56, 58, 60); at the same time the number of chromosomes in a plate is in many instances greatly reduced (Fig. 55).

The same types of modifications produced by diluted sea-water persist after the eggs have been returned to normal sea-water and in all of these cleavages, if one saw only the end result, the chromatic connections would seem to indicate that the nuclei had divided by amitosis. However a study of various stages in this process shows conclusively that this is not the case but that all of these divisions are modified forms of mitosis.

V. Conclusions.

1. The modern revival of interest in amitosis is due to a reaction against the chromosome theory. If nuclear and cell divisions ever take place by amitosis in normally developing sex

cells and embryonic cells it would deal a fatal blow to that theory. The occurrence of amitosis in fully differentiated tissue cells or in cells which do not undergo division would not affect the chromosome theory.

2. When direct division of the nucleus occurs it is rarely if ever accompanied by division of the cell body. The individual nuclear vesicles or karyomeres are not whole nuclei but fragments of a nucleus and when the cell actually divides these karyomeres combined form the typical number of chromosomes which unite into a single spindle and divide in the typical manner, as recently shown by Macklin.

3. Many apparent cases of amitosis are merely modified mitoses of which the following forms are described in this paper:

(*a*) The scattering of chromosomes and their failure to unite into a single nuclear vesicle.

(*b*) Mitotic division of the nucleus without division of the cell body and the consequent formation of binucleate or polynucleate cell.

(*c*) The failure of daughter chromosomes to pull apart in the spindle and the consequent formation of chromatic connections between daughter nuclei.

(*d*) The persistence of the nuclear membrane with division of the chromosomes by mitosis and of the nuclear vesicle by constriction.

4. There is not a single wholly conclusive case in which amitosis has been shown to occur in the division of normally differentiating cells. Therefore the attempts to disprove the chromosome theory in this way have failed.

5. Mitosis and amitosis are fundamentally unlike. Mitosis is the one and only method of bringing about equal division and distribution of the chromatic material of the nucleus. Amitosis is not a genuine divisional phenomenon at all but merely a means of increasing the nuclear surface and of distributing nuclear material throughout the cell, comparable to nuclear lobulation, fragmentation or distribution. These two processes are not equivalent or even comparable nor may one of them be converted into the other.

LITERATURE CITED.

Bonnevie, K.
'08 Chromosomenstudien I. Arch. f. Zellforsch., 1.
Boveri, Th.
'07 Zellenstudien, VI. Jena.
Calkins, G. N.
'01 The Protozoa. New York.
Child, C. M.
'07a Amitosis as a Factor in Normal and Regulatory Growth. Anat. Anz., 30.
'07b Studies on the Relation between Amitosis and Mitosis. Biol. Bull., 12, 13.
'10 The Occurrence of Amitosis in Monezia. Id., 18.
'11 The Method of Cell Division in Monezia. Id., 21.
Conklin, E. G.
'97 Relation of Nucleus and Cytoplasm in the Intestinal Cells of Land Isopods. Am. Naturalist, January, 1897.
'01 The Individuality of the Germ Nuclei during the Cleavage of the Egg of Crepidula. Biol. Bull., 2.
'02 Karyokinesis and Cytokinesis, etc. Jour. Acad. Nat. Sci. Phila., 12.
'03 Amitosis in the Egg Follicle Cells of the Cricket. Am. Naturalist, 37.
'12 Experimental Studies on Nuclear and Cell Division. Jour. Acad. Nat. Sci. Phila., 15.
Dahlgren and Kepner.
'08 Principles of Animal Histology. New York.
Foot and Strobell.
'11 Amitosis in the Ovary of Protenor belfragei, etc. Arch. f. Zellforsch., 7.
Glaser, O.
'08 A Statistical Study of Mitosis and Amitosis in the Endoderm of Fasciolaria. Biol. Bull., 14.
Godlewski, E.
'09 Das Vererbungsproblem, etc. Vorträge u. Aufsätze Entw. Mech., 9.
Gurwitsch, A.
'04 Morphologie und Biologie der Zelle. Jena.
Häcker, V.
'95 Ueber die Selbstängigkeit der väterlichen und mütterlichen Kernsbestandteile, etc. Arch. mik. Anat., 49.
'00 Mitosen im Gefolge amitosenähnlicher Vorgänge. Anat. Anz., 17.
Hargitt, C. W.
'04 The Early Development of Eudendrium. Zool. Jahrb., 20.
'11 Some Problems of Coelenterate Ontogeny. Jour. Morph., 22.
Harmon, Mary T.
'13 Method of Cell Division in the Sex Cells of Taenia teniaeformis. Jour. Morph., 24.
Heidenhain, M.
'94 Neue Untersuchungen über die Centralkörper, etc. Arch. mik. Anat., 43.
Henneguy, L.
'96 Lecons sur la Cellule. Paris.
Herbst, C.
'09 Vererbungsstudien, VI. Arch. Entw. Mech., 27.

Hertwig, R.

'98 Ueber Kernteilung, Richtungskörperbildung und Befruchtung von Actinosphaerium eichornii. Abh. Bäyer. Akad. Wiss., 19.

Jordan, H. E.

'08 The Accessory Chromosome in Aplopus mayeri. Anat. Anz., 32.

Jörgensen, M.

'08 Untersuchungen über die Eibildung bei Nephelis, etc. Arch. f. Zellforsch., 2.

Konopacki, M.

'11 Ueber den Einfluss hypertonischer Lösungen auf befruchtete Echinideneier. Arch. f. Zellforsch., 7.

Knoche, E. von.

'10 Experimentelle und andere Zellstudien am Insekten-ovarium. Zoöl. Anz., 35.

Lang, A.

'01 Lehrbuch der vergleichenden Anatomie, IIAufl. Jena.

Macklin, C. C.

'16 Amitosis in Cells growing *in vitro*. Biol. Bull., 30.

Maximow, A.

'08 Ueber Amitose in den embryonalen Geweben bei Säugetieren. Anat. Anz., 33.

Mead, A. D.

'98 The Origin and Behavior of the Centrosomes in the Annelid Egg. Jour. Morph., 14.

Morgan, T. H.

'99 The Action of Salt Solutions on the Unfertilized and Fertilized Eggs of Arbacea, etc. Arch. Entw. Mech., 8.

Moroff, Th.

'09 Oögenetische Studien. I., Copepoden. Arch. f. Zellforsch., 2.

Nathansohn, Al.

'00 Physiologische Untersuchungen über amitotische Kernteilungen. Jahrb. wiss. Bot., 35.

Nemec, B.

'03 Ueber die Einwirkung des Chloralhydrats auf die Kern- und Zell-teilung. Id., 39.

Nowikoff, M.

'10 Zu Frage nach der Bedeutung der Amitose. Arch. f. Zellforsch., 5.

Patterson, J. T.

'08 Amitosis in the Pigeon's Egg. Anat. Anz., 32.

Richards, A.

'09 On the Method of Cell Division in Taenia. Biol. Bull., 17.

'11 The Method of Cell Division in the Development of the Female Sex Organs of Monezia. Id., 20.

'17 The History of the Chromosomal Vesicles in Fundulus and the Theory of the Genetic Continuity of the Chromosomes. Id., 32.

Rubaschkin, W.

'05 Ueber doppelte und polymorphe Kerne in Tritonblastomeren. Arch. mik. Anat., 66.

Schiller, L.
'09 Ueber kunstliche Erzeugung primitiver Kernteilungsformen bei Cyclops. Arch. Entw. Mech., 27.
Strasburger, E.
'08 Chromosomenzahlen, Plasmastructuren, Vererbungsträger und Reductionsteilung. Jahrb. wiss. Bot., 45.
Vejdowsky, Fr.
'12 Zum Problem der Vererbungsträger. Konigl. Böhm. Gesell. der Wiss. Prag.
Wasielewski, W.
'02, '03 Theoretische und experimentelle Beiträge zur Kentniss der Amitose, I., II. Jahrb. wiss. Bot., 38, 39.
Wenrich, D. H.
'16 The Spermatogenesis of Phrynotettix magnus, etc. Bull. Mus. Comp. Zoöl., 60.
Young, R. T.
'13 The Histogenesis of the Reproductive Organs of Taenia pisiformis. Zoöl. Jahrbücher, 35.

Description of Figures.

Figs. 1–60 represent whole eggs of *Crepidula plana* which had been fixed, stained and mounted. All figures were drawn with camera lucida under Zeiss apochromatic objective 3 mm. Hom. Im., Comps. ocular 4. As drawn they represent a magnification of 333 diameters; in the process of reproduction they have been reduced about one third.

Figs. 1–18 represent eggs which had been subjected to increased temperature.

Explanation of Plate I.

Fig. 1. No. 1174(2). 37°, ¼ hr., during second maturation; then kept at room temperature (ca. 27°) for 3 hrs. The first polar body is shaded by transverse lines; the sperm nucleus is shown at the left; the other nuclei are karyomeres formed by the scattering of chromosomes of the second maturation mitosis.

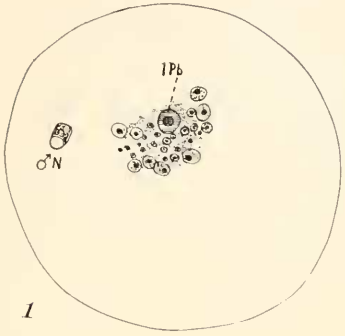
Fig. 2. No. 1175(2). 33°, ¼ hr., during first cleavage; room temperature (ca. 27°) 3 hrs. The imperfect fusion of the karyomeres (gonomeres?) gives an appearance of amitosis to each of the nuclei.

Fig. 3. No. 1176(2). 34°–35°, ¼ hr., during second cleavage; room temperature (ca. 23°) 3 hrs. Karyomeres formed by scattering of chromosomes of second cleavage.

Fig. 4. No. 1173(2). 37°, ¼ hr., beginning of third cleavage; room temperature (ca. 27°) 3 hrs. Many karyomeres formed by scattering of chromosomes of third cleavage. Traces of centrospheres are shown in the cells.

Fig. 5. No. 1176(2). 34°–35°, ¼ hr., during third cleavage; room temperature (ca. 23°) 3 hrs. Karyomeres formed by scattering of chromosomes of third cleavage. In one quadrant a micromere was formed, in the other quadrants cell division was suppressed. Traces of centrospheres as in preceding.

Fig. 6. No. 1173(2). 37°, ¼ hr., during anaphase of third cleavage; room temperature (ca. 27°) 3 hrs. In two quadrants micromeres have formed. Daughter nuclei are elongated in the greater dimensions of the chromosome plates, the latter having fused together into irregular masses.



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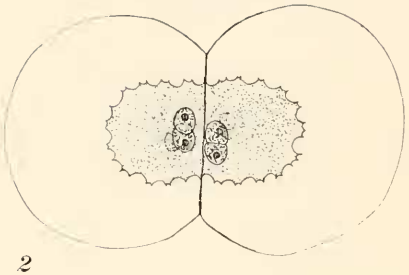

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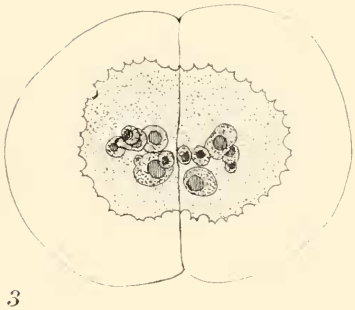

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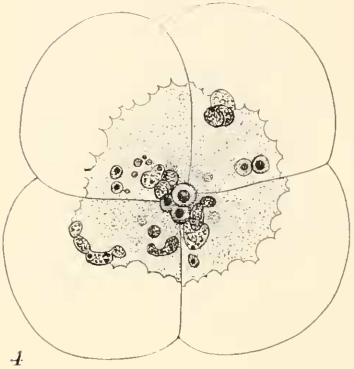

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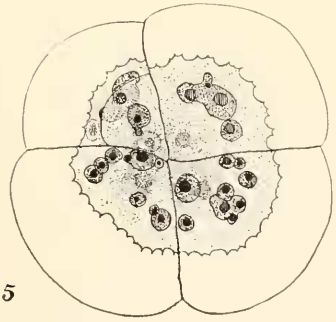

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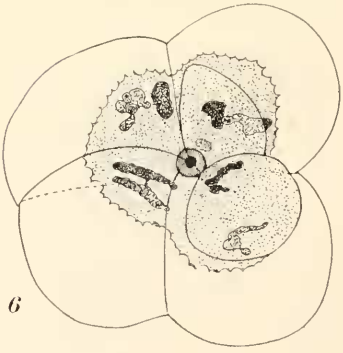

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Explanation of Plate II.

Fig. 7. No. 960. Ca. 35°, 4 hrs., during third cleavage. The chromosomes have scattered and formed numerous chromosomal vesicles.

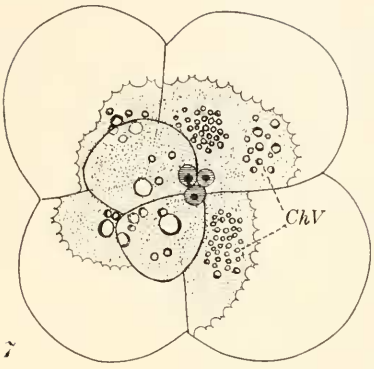
Fig. 8. No. 1176(2). 34°–35°, ¼ hr., during anaphase of third cleavage; room temperature (ca. 23°) 3 hrs. Many nuclei are lobed or irregular; nuclei are most abnormal in the quadrant in which a micromere did not form, and in which mitosis was not so far advanced at the time of the experiment, as in the other quadrants.

Fig. 9. No. 1176(1). 34°–35°, ¼ hr., during fourth cleavage of macromeres. In three quadrants the chromosomes are scattered in the spindles, in the fourth are chromosomal vesicles. The nuclei and centrosomes in the first set of micromeres are in a resting stage and are absolutely normal.

Fig. 10. No. 1176(1). 34°–35°, ¼ hr., during fourth cleavage. Chromosomal vesicles are present in one of the macromeres, and chromosomes are more or less scattered in all micromeres of the first set.

Fig. 11. No. 1176(2). 34°–35°, ¼ hr., during fourth cleavage; room temperature (23°) 3 hrs. The nuclei formed after the division of the first set of micromeres are lobed and irregular; the scattered chromosomes in the macromeres have formed many karyomeres. This figure shows an egg like Fig. 9 after being kept three hours at normal temperature.

Fig. 12. Same slide as preceding. Many lobulated nuclei and karyomeres in the cells derived from the first set of micromeres; nuclei in second set of micromeres and in macromeres are nearly normal. This figure shows an egg like Fig. 10, after being kept three hours at normal temperature.



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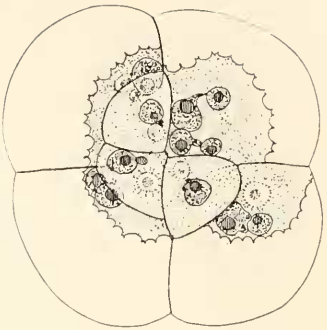

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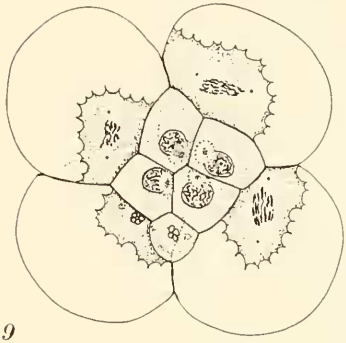

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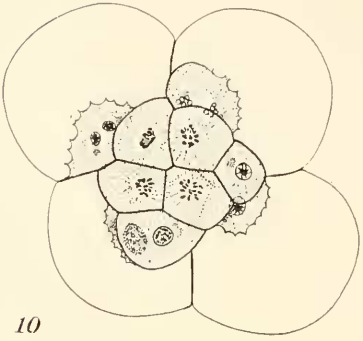

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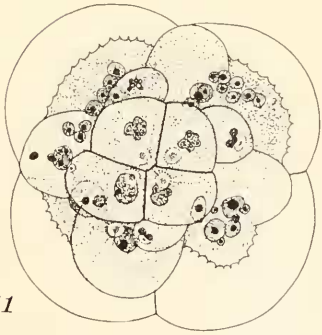

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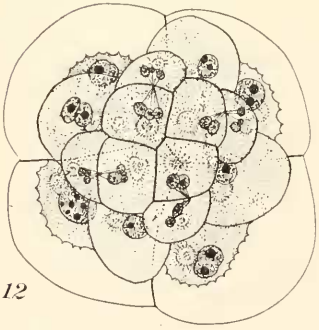


12

Explanation of Plate III.

Figs. 13–18 were subjected to temperature so high (37°), or for so long a period (35° for four hours) that further cell division was stopped in almost all cases, though the protoplasm remained transparent and apparently alive.

Fig. 13. No. 1171(2). 37°, ½ hr. during first cleavage; room temperature (ca. 25°) 15 hrs. Astral areas are large and distinct; chromosomes are widely scattered or clumped in two principal masses outside the astral areas and at the surface of the egg; chromosomes do not become vesicular.

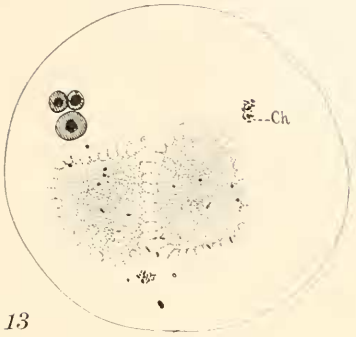
Fig. 14. Same slide as preceding; subjected to heat during second cleavage, and showing results similar to preceding.

Fig. 15. No. 960. Ca. 35°, 4 hrs., during resting 2-cell stage. The plasma is much contracted and vacuolated and the chromatin of the nuclei is in the form of hollow spheres, which look like chromosomal vesicles.

Fig. 16. Same slide as preceding; heated during second cleavage mitosis; plasma shrunken and chromosomes in the form of vesicles.

Fig. 17. No. 1171(2). 37°, ½ hr., during fourth cleavage; room temperature (ca. 25°) 15 hrs. Chromosomes in the macromeres are clumped together at the surface of the egg, and are outside the plasma areas. There is a triaster in one of the micromeres, while the others appear normal.

Fig. 18. Same slide as preceding; 24-cell stage. All centrospheres instead of being at the surface, as in normal eggs, are at the deeper ends of the cells next to the spacious segmentation cavity.



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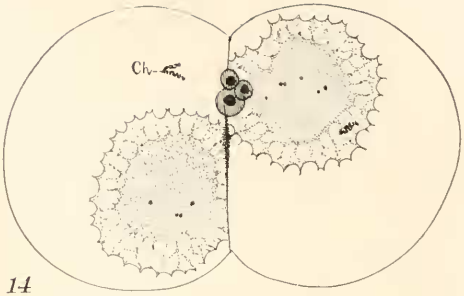

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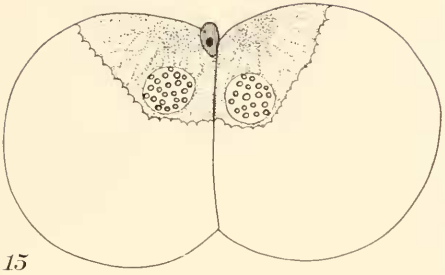

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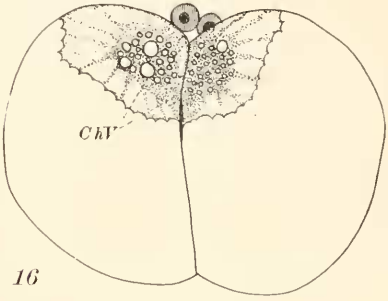

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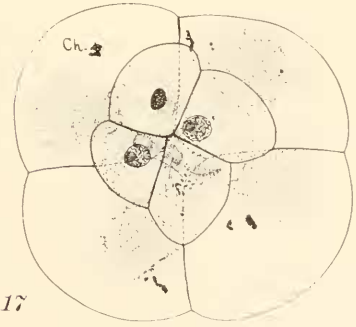

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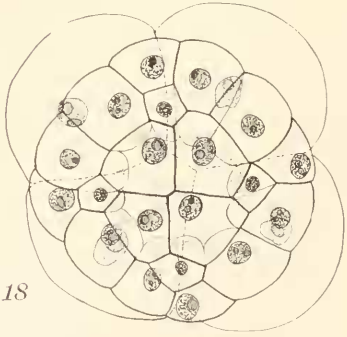


18

Explanation of Plate IV.

Figs. 19–48 (with exception of Fig. 30) represent eggs which had been subjected to hypertonic solutions.

Fig. 19. No. 822. 2 per cent. NaCl, 16 hrs., normal sea-water 8 hrs. Numerous karyomeres were formed after maturation and before the first cleavage, presumably during the first cleavage mitosis.

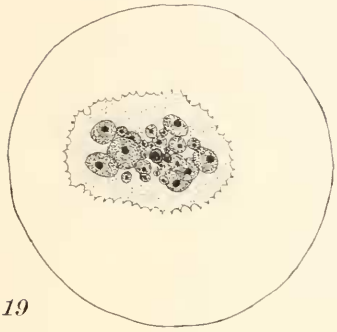
Fig. 20. Same slide as preceding. Karyomeres formed at the close of the first cleavage by chromosomes failing to unite. Centrospheres (*s*) unusually large.

Fig. 21. No. 837. $\frac{3}{4}$ per cent. KCl 9 hrs.; normal sea-water 35 hrs. Karyomeres irregular in size, shape and distribution.

Fig. 22. Same slide as preceding, with similar karyomeres.

Fig. 23. No. 822. 2 per cent. NaCl 16 hrs.; normal sea-water 8 hrs. Second cleavage abnormal, with karyomeres in two of the cells.

Fig. 24. No. 814. 2 per cent. NaCl 1 hr.; normal sea-water 17 hrs. Third and fourth cleavages abnormal, with karyomeres in most of the cells.



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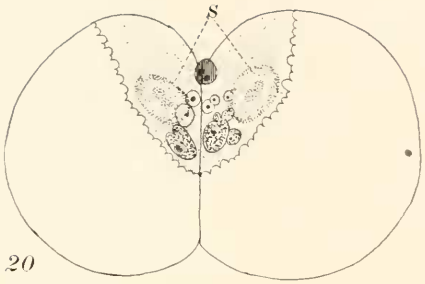

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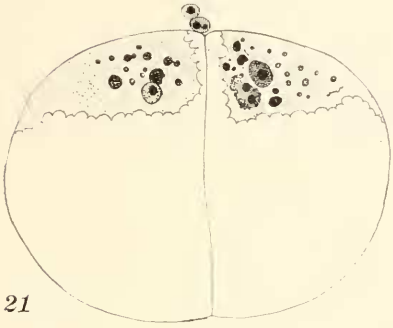

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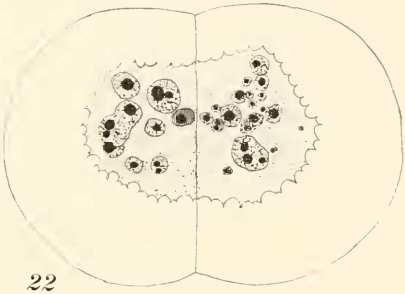

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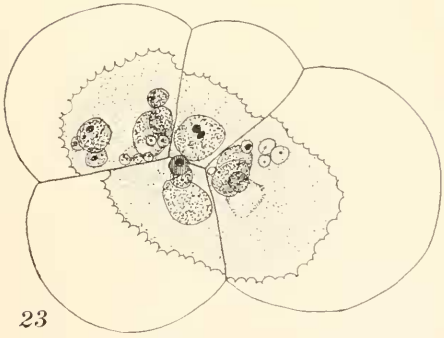

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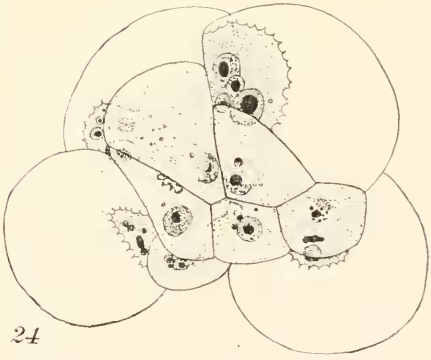

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Explanation of Plate V.

Fig. 25. No. 927. 2 per cent. NaCl 16 hrs., at close of third cleavage; normal sea-water 24 hrs. Numerous karyomeres in the cells.

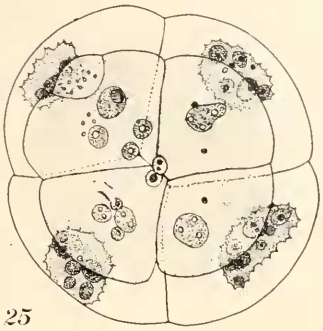
Fig. 26. No. 867. 8 per cent. $MgCl_2$ $\frac{3}{4}$ hrs., during third cleavage; normal sea-water $6\frac{1}{2}$ hrs. Numerous karyomeres in resting cells; in dividing cells, polyasters and scattered chromosomes.

Fig. 27. No. 972, same slide as Fig. 25. Karyomeres in all resting cells; polyasters and scattered chromosomes in dividing ones.

Fig. 28. No. 971. 2 per cent. NaCl 16 hrs., during third cleavage; normal sea-water 12 hrs. Similar to preceding.

Fig. 29. No. 828. 1 per cent. NaCl 2 hrs., during third cleavage; normal sea-water $6\frac{1}{2}$ hrs. Polyasters and chromatic connections between daughter nuclei.

Fig. 30. No. 956. Sea-water diluted with equal parts of fresh water 2 hrs.; normal sea-water 36 hrs. The nuclei in yolk-containing cells show many karyomeres.



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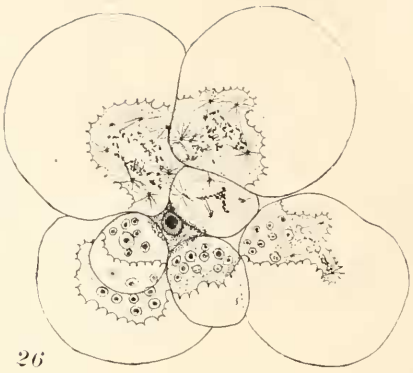

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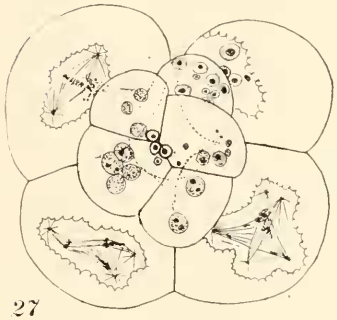

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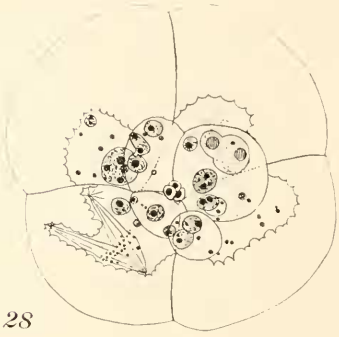

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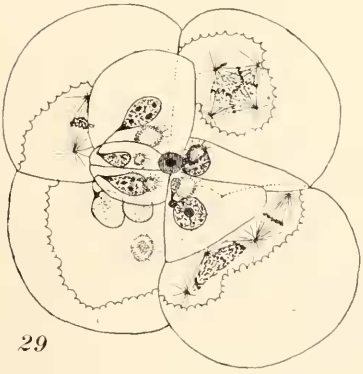

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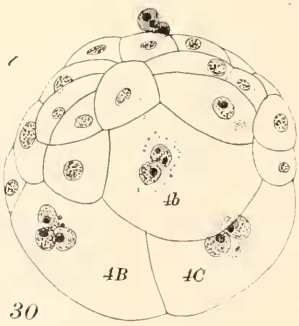

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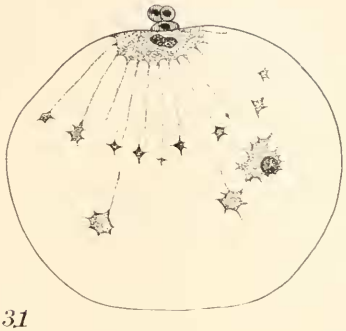
Explanation of Plate VI.

Fig. 31. Normal egg showing cytasters sometimes present in eggs at the stage between the close of the maturation divisions and the beginning of the first cleavage. These cytasters are local aggregations of plasma along the lines of astral radiations.

Figs. 32, 34, 35. Eggs from Exp. No. 805. 2 per cent. NaCl 4 hrs. Numerous cytasters are shown in different cell stages and division phases; in every case these cytasters are local aggregations of plasma along the lines of astral radiations, the remaining plasma being gathered around the nuclei or spindles.

Figs. 33, 36. No. 998(3). 1 per cent. NaCl 5 hrs. The plasma is concentrated around the spindles, only small portions being left along the astral radiations in the 2-cell stage and none in the 4-cell stage. No cytasters are present after the 2-cell stage.



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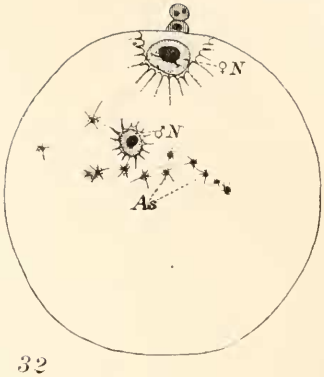

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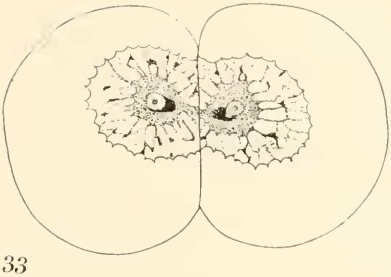

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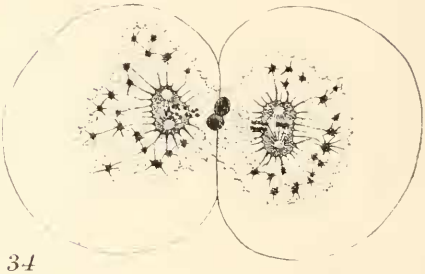

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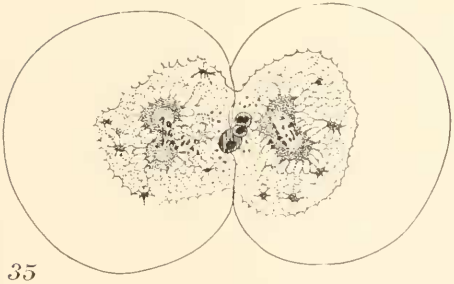

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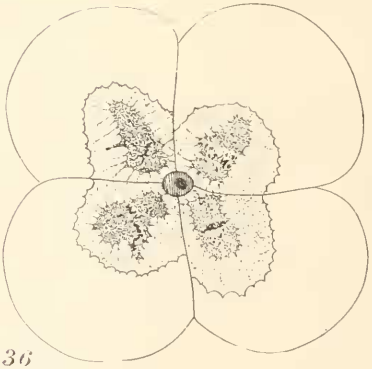

36

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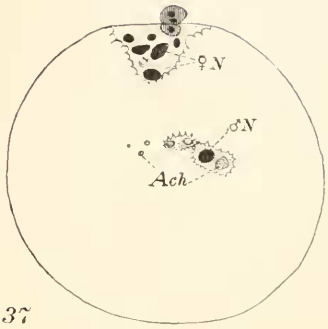
Explanation of Plate VII.

Figs. 37–42. Various eggs from one experiment, viz., No. 809. 2 per cent. NaCl $15\frac{1}{2}$ hrs. All figures show the chromatin in a densely chromatic mass and in all cases except Fig. 37, where the egg nucleus consists of several karyomeres, all the chromatin of each nucleus is aggregated into a single mass. On the other hand the achromatin consists of vesicles of different sizes which are more or less widely scattered, though a large achromatic vesicle is usually found adjoining a chromatic mass. This achromatin has not been squeezed out of the chromatic mass by the action of the salt solution, but the chromosomes of the daughter nuclei have been prevented from absorbing achromatin by the action of the salt solution.



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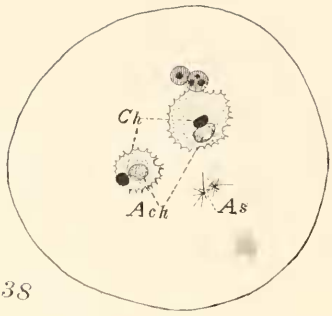

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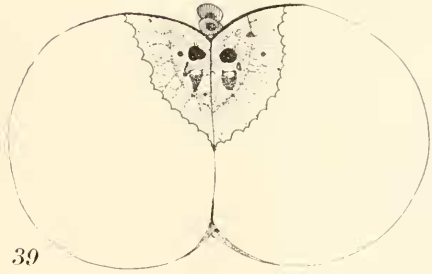

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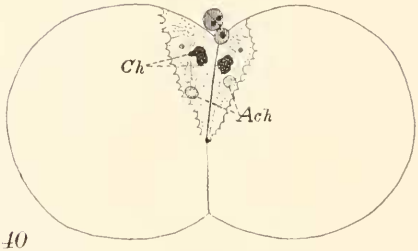

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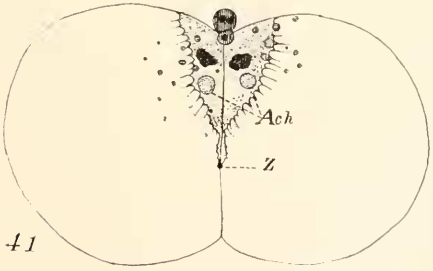

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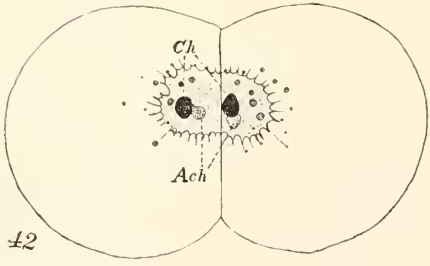
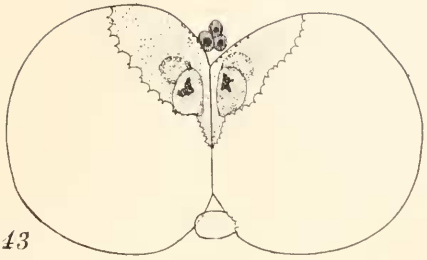


42

Explanation of Plate VIII.

Figs. 43–48 are different eggs from the same experiment, viz., No. 810. 3 per cent. NaCl $15\frac{1}{2}$ hrs. All the eggs were in the anaphase or telophase of the second cleavage at the time they were placed in the salt solution, and in all cases the chromosomes have remained in the form of a dense chromatic plate or mass which has not become vesicular. In Fig. 45, the spindle remnants between the chromosomal plates has become vesicular; in Figs. 46 and 47, the spindle area has become an elongated achromatic vesicle, within which lies the dense chromatic mass; in Figs. 43, 44, 48 the achromatic vesicle has become more nearly spherical in outline, while the chromatic mass is not quite so dense as in earlier stages. These figures show that when the chromosomes are prevented from absorbing achromatin and becoming vesicular nuclei, the achromatin of the spindle region may become a vesicle by the formation of a delicate achromatic membrane around itself.



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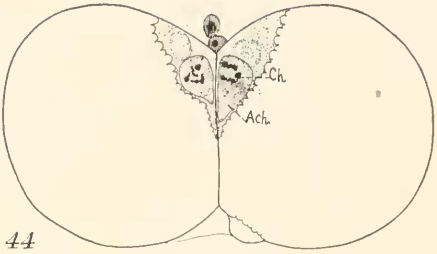

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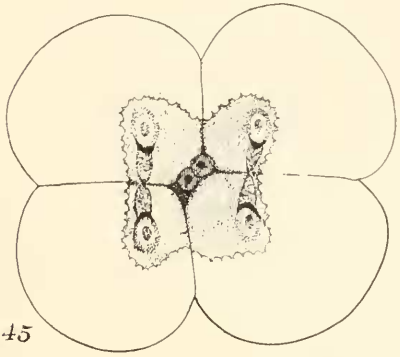

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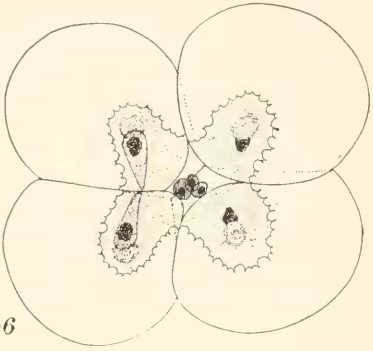

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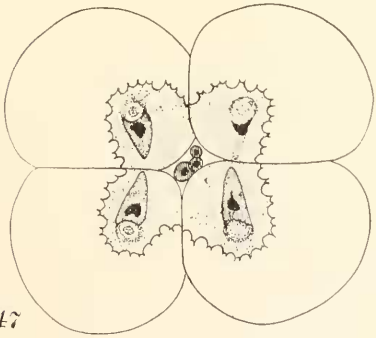

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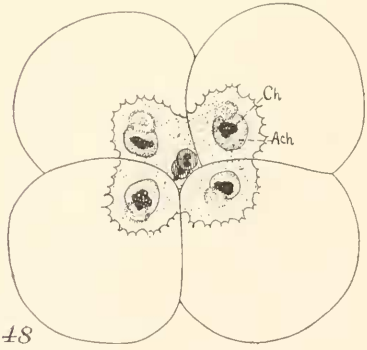


48

Explanation of Plate IX.

Figs. 49–60 represent eggs which had been subjected to diluted sea-water.

Figs. 49–54. No. 859. Sea-water 1 part, fresh water 2 parts, 1 hr.; normal sea-water 4 hrs. Eggs were treated with this diluted sea-water during the third and fourth cleavages, thus causing a scattering or stretching out of chromosomes along the spindle and the formation of chromatic connections between daughter nuclei. In cell D, Fig. 49, the division was stopped in the prophase, the nucleus being pear-shaped with the chromatin chiefly in the narrow upper end of the pear. It is significant that the long axis of the pear is in the direction of the spindle axis and that if the constriction were to separate the neck from the body of the pear, the daughter nuclei thus formed would be of approximately the same size as in normal eggs.

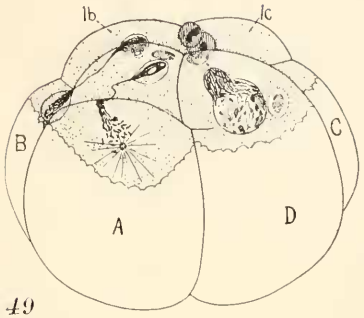
Fig. 51 shows more advanced stages of a similar process in which the chromatin is chiefly in the smaller, upper nuclei, the achromatin in the larger, lower ones.

Figs. 49 and 50 show certain nuclei in which the nuclear membrane remains intact though the chromosomes are arranged along a line or spindle connecting the two centrosomes.

Figs. 52–54. Eggs in which the third cleavage took place by a modified form of mitosis which left chromatic connections between daughter nuclei, and yet typical spindles for the fourth cleavage are present in some of the macromeres.

Fig. 52. In all four quadrants of this egg nuclear division at the third cleavage took place by modified mitosis, the daughter nuclei remaining connected by chromatic threads; in only two quadrants were micromeres formed, and the macromeres of these quadrants are now dividing by mitosis.

Figs. 53, 54. All daughter nuclei are connected by chromatic threads. Chromatin aggregates on the side of the nucleus next the centrosome (Fig. 54, *D*) and the chromatic connections between daughter nuclei run to the outer sides of the nuclei and spindles in the macromeres; the latter may be approximately normal, though the spindles may be out of proper position and the chromosomes more or less scattered.



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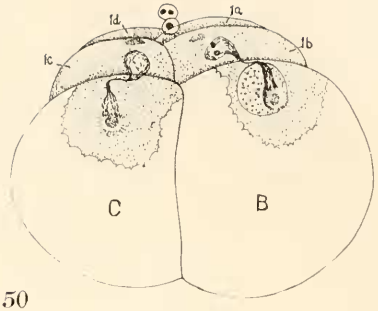

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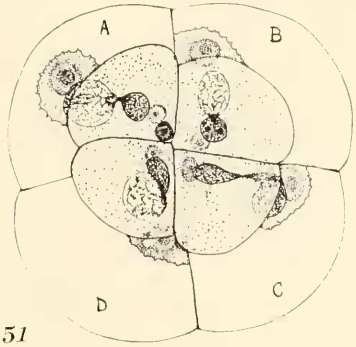

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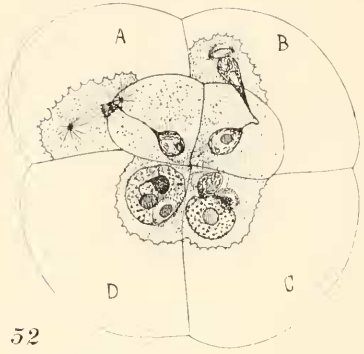

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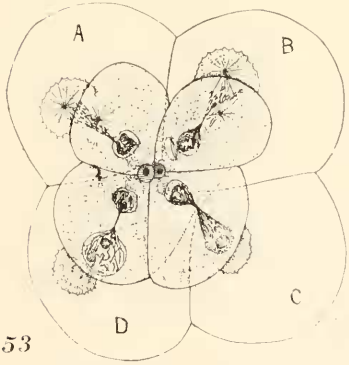

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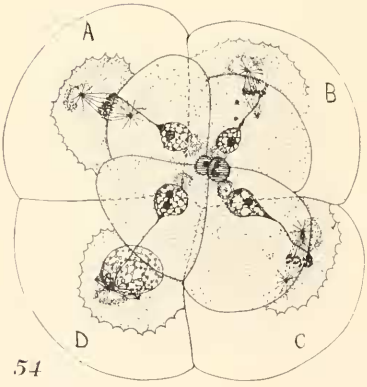

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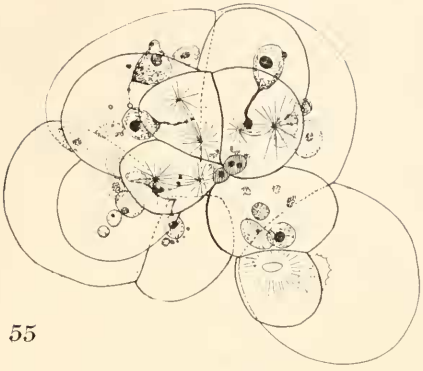
Explanation of Plate X.

Figs. 55–60. All from one experiment, No. 858. Eggs in 2–4-cell stage were left in sea-water diluted with equal parts of fresh water for two hours; then in normal sea-water fourteen hours. Remains of chromatic connections which were formed during the third cleavage are seen in Figs. 55, 56, 60. Other chromatic connections which were formed in later cleavages after the eggs had been returned to normal sea-water are shown in Figs. 57–60.



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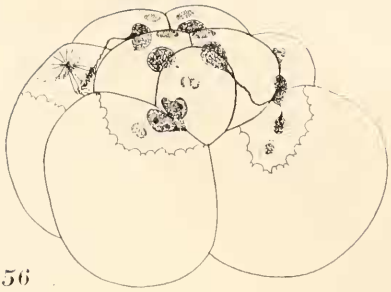

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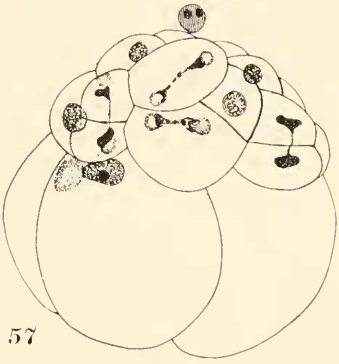

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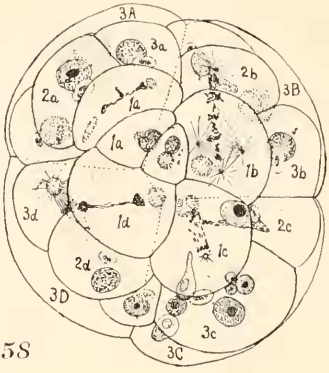

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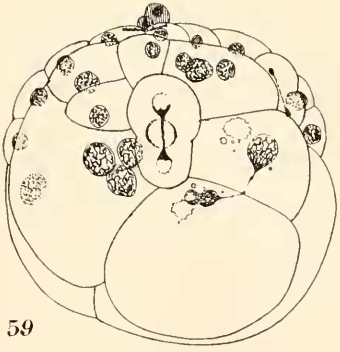

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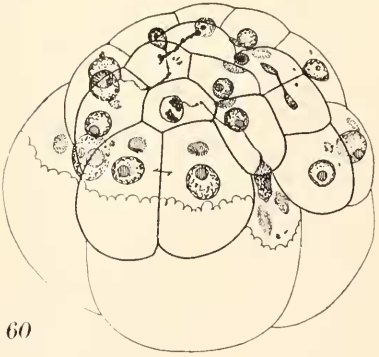

60

THE INFLUENCE OF GENERAL ENVIRONMENTAL CONDITIONS ON THE PERIODICITY OF ENDOMIXIS IN PARAMECIUM AURELIA.

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(Twelve figures.)

It is clear from the evidence submitted in previous papers that there are normal, minor, periodic fluctuations (rhythms) in the rate of reproduction of *Paramecium*,[1] and that at the low point in the division rate between two rhythms there normally occurs an intracellular reorganization process (endomixis).[2] The data previously presented also show that in *Paramecium aurelia* endomixis occurs, generally speaking, at intervals of about four weeks or about fifty generations, and that the synchronism of the process in different lines of the same race under identical conditions is remarkably exact.[3] But, as was stated incidentally, on the basis of our experience in working out the cytological phenomena of endomixis: "It is possible to retard or hasten the occurrence of the process by the character of the culture medium. For example, it may occur a few days earlier in animals not supplied daily with fresh culture fluid than in the regular lines."[4] Such being the case it is important to determine the influence of environmental conditions on the duration of the rhythms and the occurrence of endomixis.

The present paper comprises chiefly a study of the effects of what may be termed general changes in the culture conditions,

[1] Woodruff and Baitsell, "Rhythms in the Reproductive Activity of Infusoria," *Journ. Exper. Zoöl.*, XI., 4, 1911.

[2] Woodruff and Erdmann, A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Journ. Exper. Zoöl.*, XVII., 1914; Erdmann and Woodruff, The Periodic Reorganization Process in *Paramecium caudatum*," *Journ. Exper. Zoöl.*, XX., 1916; Woodruff, "Rhythms and Endomixis in Various Races of *Paramecium aurelia*," BIOL. BULL., XXXIII., 1917.

[3] Cf. Woodruff and Erdmann, 1914, Tables 1, 2, 3.

[4] Woodruff and Erdmann, 1914, p. 485.

such as markedly different culture media and temperatures on rhythms and endomixis. It seemed important to obtain a more definite background of knowledge of the influence of what perhaps may be called normal environmental changes before attempting to study the influence of, for example, specific chemical agents on the process.

It is assumed in the present paper that the reader is familiar with the earlier work on *Paramecium* which has been published from the Yale Laboratory.

Material and Methods.

The organisms employed in the work were from pedigreed cultures of *Paramecium aurelia*. Some of these had been under culture conditions for long periods—one for more than 5,000 generations—while others were started with this work in mind. Each of the five races used was started originally with a 'wild' individual which was secured from a locality far removed from that of the others, so that representative diverse material was afforded. The early life history of each of these cultures has been presented in connection with other work and the reader is referred to these papers for further details.[1]

All the organisms studied have been carried in pedigreed subcultures isolated from the respective main cultures of the various races, and since the method of conducting such cultures has been described many times in earlier papers it need not be repeated in detail here. Suffice it to say that the method involves the isolation of one or more animals from each line of every subculture practically every day and in addition, for the work in hand, the preservation and cytological study of some of the stock animals left over at the time of the daily isolations. In this way the occurrence of endomixis has been determined.

The main cultures have been carried on the 'varied' culture medium which we have found for ten years so favorable in breeding *Paramecium*.[2] This consists of infusions of vegetable and animal debris collected from time to time from laboratory

[1] Cf. especially Woodruff, Biol. Bull., XXXIII., 1917.

[2] Woodruff: "The Life Cycle of *Paramecium* when Subjected to a Varied Environment," *American Naturalist*, XLII., 1908.

aquaria, ponds, etc., and, of course, thoroughly boiled and allowed to attain room temperature before being used. Some of the subcultures directly involved in the experiments have been bred on this medium. Others have been bred on the beef-extract medium which we have employed in other work on *Paramecium*,[1] or on other media which will be described in connection with the individual experiments.

The subcultures which have been the basis of the present work may be tabulated as follows:

SUBCULTURES.

A (from Main Culture I) Oct. 15, 1914, to Feb. 12, 1916. (485 days.)
4675th to 5592d generations.

AE (from IE)[2] Oct. 15, 1914 to Aug. 12, 1915. (Twice restarted during the 300 days.)
4637th to 5079th generations.

O (from III) Oct. 15, 1914 to Nov. 20, 1915. (Once restarted during the 400 days.)
17th to 775th generations.

B (from IV) Jan. 8, 1915 to Jan. 14, 1916. (372 days.)
3d to 609th generations.

M (from V) July 17, 1915 to Feb. 23, 1916. (222 days.)
3d to 550th generations.

W (from VI) Aug. 12, 1915 to Jan. 14, 1916. (152 days.)
3d to 302d generations.

The data are presented chiefly by graphs of the division rate of the various subcultures. These are plotted by averaging the daily rate of division of the several lines of the respective subcultures and then again averaging this for five-day periods. The figures 1, 2, 3 represent divisions and 10, 20, etc., indicate the number of the five-day periods. An *E* shows that endomixis was observed during the five-day period. Inclusion of a part of the curve within brackets indicates that the cells were not studied cytologically during this time. Cf. Figs. 1 and 7. Since so much depends on these graphs and the five-day periods which they comprise it may be well to repeat a statement made in a previous paper:[3]

[1] Woodruff and Baitsell, "The Reproduction of *Paramecium aurelia* in a 'Constant' Culture Medium of Beef Extract," *Journ. Exper. Zoöl.*, XI., 1, 1911.

[2] *IE* is a subculture isolated from Main Culture I. in October, 1913.

[3] Woodruff and Erdmann, *Journ. Exper. Zoöl.*, 1914, p. 477.

"The five-day period was adopted in the presentation of our results because this was the method of constructing the

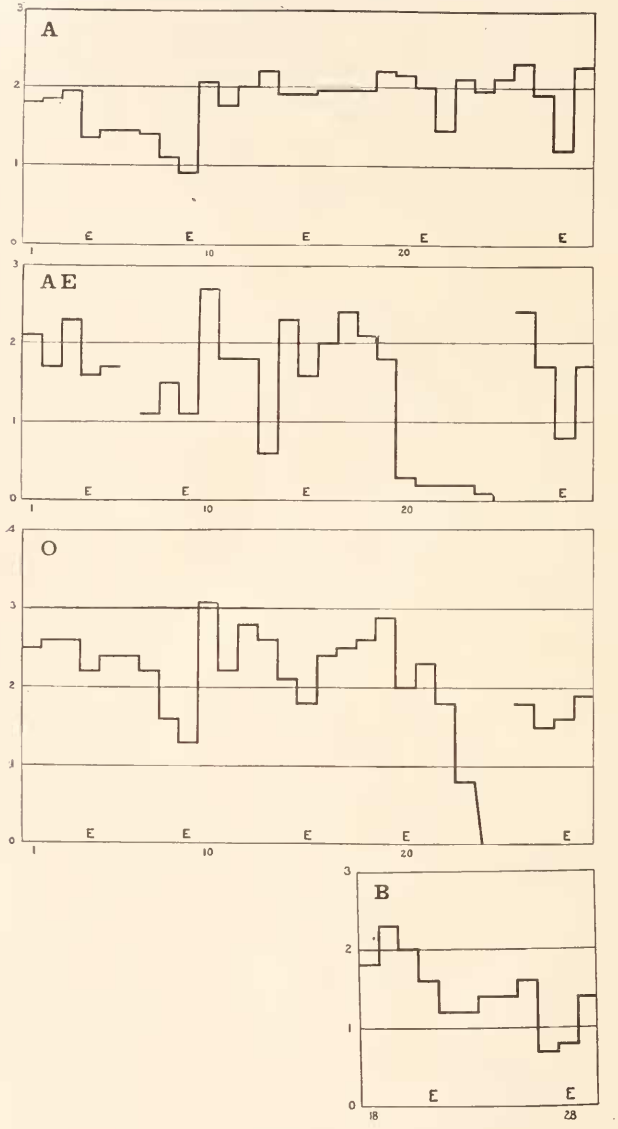

FIG. 1.

graph emphasized in the original study of rhythms in this culture.[1] It is realized, of course, that a five-day period is

[1] Woodruff and Baitsell, *Journ. Exper. Zoöl.*, XI., 1, 1911.

largely an arbitrary one and that the ideal graph would present the momentary changes in the metabolism of the cell. Data for such a curve being absolutely impossible to secure, it might seem at first glance that the daily record of division would approach most nearly to this ideal condition. As a matter of fact, the twenty-four-hour period is as arbitrary as the five-day period when it is considered that this is a long period when compared with the metabolic changes in the cell and that the daily record, made at approximately 11 A.M., would merely give the divisions actually completed during the previous twenty-four hours. For example, let us assume that, at the time of isolation, two animals are present, representing one division during the previous twenty-four hours. The record for that day is one division. One animal is then isolated and it divides within an hour and each of the resulting cells again divide twice before the next isolation. The record for this second day is three divisions, thus the record for the two days shows a different division rate for each day, *i. e.*, one division against three divisions, whereas a more true, but not a perfect, picture of the state of affairs is given by the statement that four divisions occurred in forty-eight hours. One might follow this argument to its logical conclusion and assume that the best method of presentation would be to average for considerable periods, *e. g.*, 10 or 30 days, but this obviously would tend to obliterate any fluctuations in the rate which are not of relatively long duration. The adoption of the five-day period was made in recognition of both of these contingencies, and it was of a duration particularly well suited to show the effect of the process on the reproductive rate, because the process extends over about nine cell divisions or a period of about six days. Consequently the effect of the process makes itself evident in the five-day plot. Certain apparent irregularities in the coincidence of the phenomena are, from an actual study of all the data at hand, clearly due to the fact that the five-day period is not ideal."

Experiments—Series I.

The experiments of Series I. may be outlined as follows:

A. Study of the periodicity of rhythms and endomixis in

different races of *Paramecium aurelia* when bred under the same *varied* culture conditions.

B. Study of the periodicity of rhythms and endomixis in

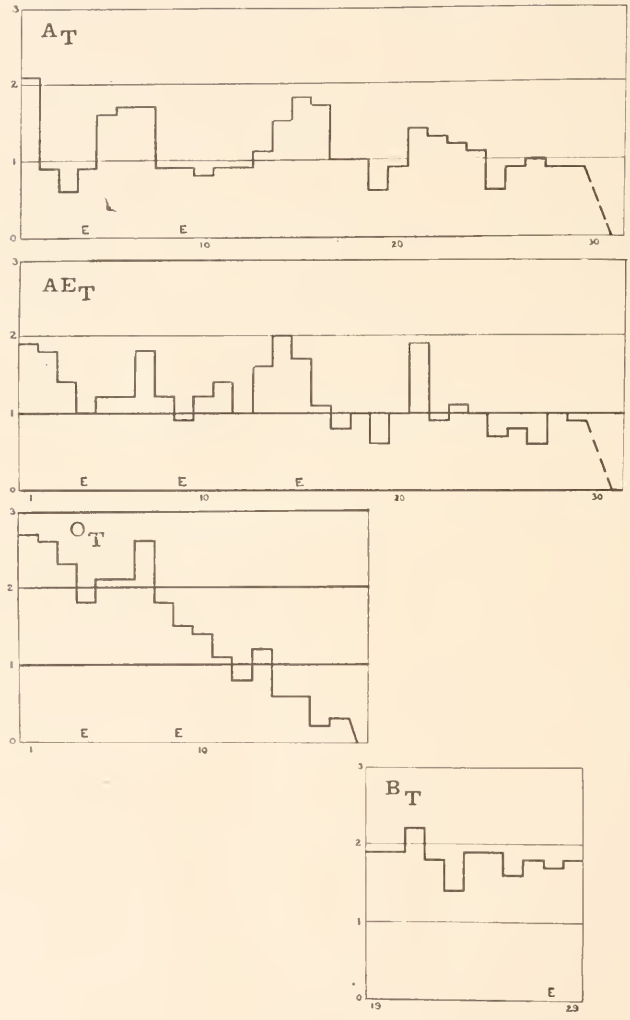

FIG. 2.

different races of *Paramecium aurelia* when bred under the same *constant* culture conditions.

C. Study of the periodicity of rhythms and endomixis in the *same* races of *Paramecium aurelia* when bred under the *varied* and under the *constant* culture conditions.

These experiments were carried on from October 15, 1914, to March 10, 1915; a period of 145 days. The subcultures employed were *A*, *AE*, and *O* throughout the work and *B* from its isolation on January 8, 1915, to the end. Each of these subcultures represents a different race of *Paramecium aurelia*, except *A* and *AE*, both of which were originally derived from the same stock, Main Culture I., about 1,000 generations before.

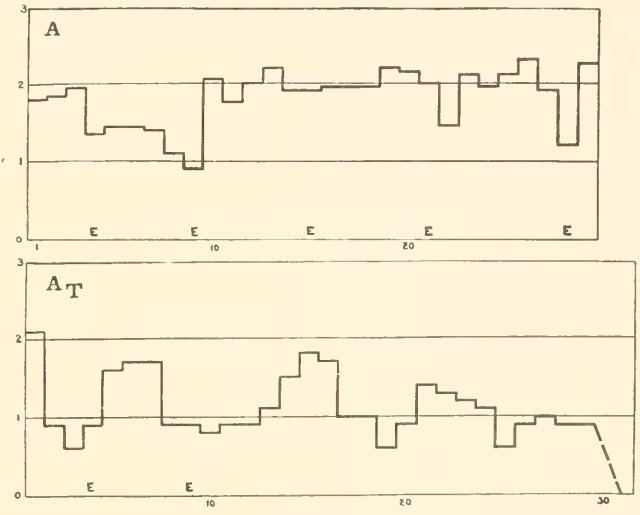

FIG. 3.

All of these subcultures were subjected to a varied culture medium and to the ordinary fluctuation in room temperature.

Sister subcultures (designated *At*, *AEt*, *Ot* and *Bt*), were isolated line by line from the above cultures at the start of the experiment and were subjected to a constant culture medium of beef extract. The temperature was maintained relatively constant at about 26° C. in a thermostat.

A.

Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same *varied* culture conditions.

Fig. 1 presents the graphs of the division rate of subcultures *A*, *AE*, *O* and *B* throughout this experiment. A study of the

figure shows that *A* underwent endomixis at periods Nos. 4, 9, 15, 21 and 28. *AE* showed endomixis at period 4 and then was lost by an accident. A new *AE* was started by isolating line by line from *AEt* (which had been branched from it 25 days before and subjected to the constant culture conditions).

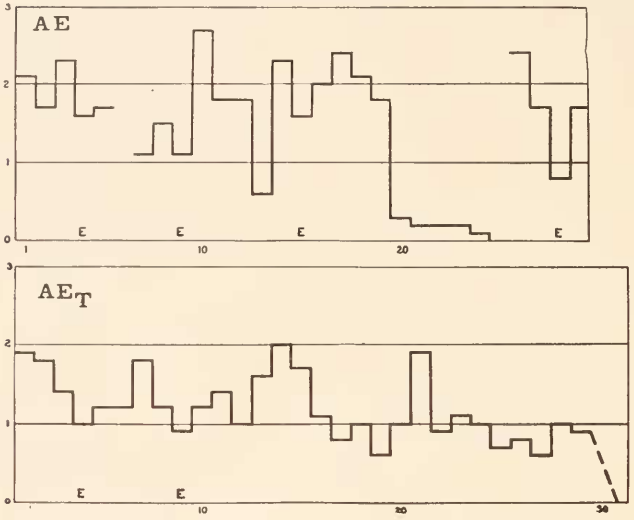

FIG. 4.

AE then showed endomixis at the periods 9 and 15 and died out in period 25 without again undergoing endomixis. Still another *AE* was isolated immediately from *AEt* and had endomixis during the 28th period. A similar survey of the graph of *O* shows that endomixis took place at periods 4, 9, 15 and 20. Subculture *O* died at period 25 from causes unknown. It was restarted from the main culture of this race at once and had endomixis at period 28. Subculture *B* was begun in period 18, that is immediately on the isolation of the race from 'wild' material. Endomixis occurred at periods 21 and 28.

Tabulation of these data shows that endomixis occurred in these subcultures as follows:

A at periods.....	4	9	15	21	28
AE " "	4 lost	9	15	0 died	28
O " "	4	9	15	20 died	28
B " "	–	–	—	21	28

This experiment, in which different races were bred under the same varied culture conditions, shows an almost perfect synchronism of endomixis in all the races; that is, in *A* bred throughout without interruption on the varied medium; in *O* bred until its death near the end of the experiment on the same medium and again in the new *O* isolated from the main culture; in *B*, which was isolated from 'wild' material during the progress of the work; and finally in *AE*, which was restarted twice from a sister culture bred under "constant" environmental conditions.

The most reasonable conclusion, on the basis of this experiment, to account for the fact that the different races immediately showed endomixis synchronously, is that the general culture conditions initially influenced the appearance of the process; that is, brought about its consummation a few days earlier or later than it would have appeared under the former environment of the races, and that, once established, the rhythmic period characteristic of the species persisted and maintained the synchronism of endomixis. If this conclusion is justified then it must be assumed that the synchronism of *B* is due to the chance isolation of this race at just the same period in the rhythm which *A*, *AE* and *O* were at at the time, or so near this period that the environmental change immediately made it coincide with that of the other races. The alternate hypothesis would be that there is in all races of this species a definitely established synchronism which holds under all normal environmental conditions. But such a theory would require more than one series of experiments to render its discussion profitable!

B.

In this set of experiments a study was made of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same practically constant culture conditions, as already described. The results are shown graphically in Fig. 2.

Subcultures *At* and *AEt* (both derived from the same race, I, about 1,000 generations previously) showed endomixis in preserved specimens at periods 4 and 9 and *AEt* also at period 15. Neither culture again underwent the process during a

period of over 100 days before it died, though the characteristic 'rhythms' in the division rate are apparent during this time. It is possible that the process did occur at about periods 19 and 25 (cf. Fig. 2) and was overlooked, but I believe that this is highly improbable in view of the thoroughness of the search.

Subculture *Ot* underwent endomixis at periods 4 and 9 and then died without repeating endomixis at the next expected period (cf. Fig. 2).

Subculture *Bt*, as the graph shows, had endomixis at the 28th period, just before the experiment was concluded. From the character of the curve it would be expected at period 23, but it was not observed.

The endomictic periods of the four subcultures (3 races) of *Paramecium* in this experiment may be tabulated as follows:

At at periods.....	4	9	0	0 died
AEt " "	4	9	15	0 "
Ot " "	4	9	0	– "
Bt " "	–	–	–	28

These experiments show three points of considerable interest. In the first place they corroborate, for races bred under constant culture conditions, what was found in the same races when bred under varied culture conditions in the experiment already described. That is, *At*, *AEt* and *Ot* show a perfect synchronism of endomixis, and this is most reasonably explained by assuming that the general culture conditions, at least initially, influenced the appearance of endomixis and that, once established, the rhythmic period characteristic of the organism persisted. *Bt* affords no data for comparison with the other races of this set of cultures, as they had ceased to show endomixis before *Bt* was started.

A second point of importance is that the 'rhythms' in the division rate are to a certain extent independent of endomixis—that is of the definitive series of nuclear phenomena—because the rhythms persist for a while in the absence of the morphological changes. One may suggest that the rhythms in the culture are an expression of the physiological conditions antecedent to the definitive onset of the nuclear changes—in other

words that the cell has undergone the preliminary stages of endomixis which ordinarily call forth the observable nuclear changes but that in the cases in hand the latter were never realized. This is equivalent to making the term endomixis coextensive with the term rhythm—the term rhythm denoting the physiological effect as indicated in the reproductive activity, while the term endomixis covers all the underlying physiological

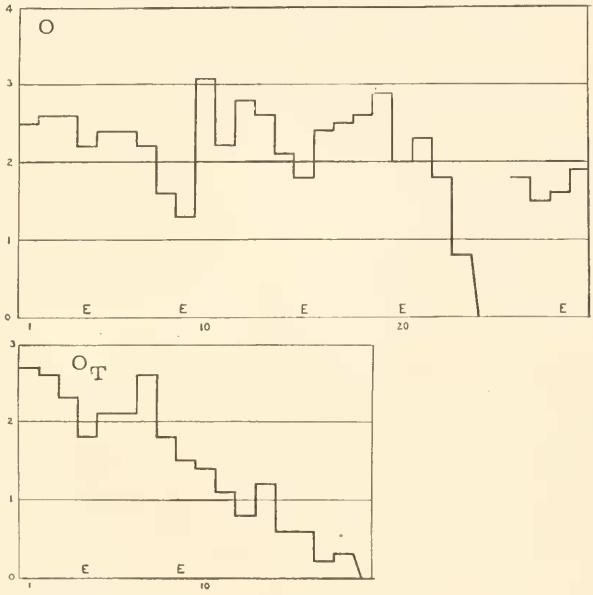

FIG. 5.

changes together with the definitive nuclear phenomena characteristic of the low point in the division rate. The term endomixis was *not* used in that broad sense when first employed by Woodruff and Erdmann and it is unprofitable to extend it now. Applying the term strictly to the complicated reorganization process of the cell does not deny the undoubted fact that these changes are but the expression of the climax of a series of physiological phenomena which probably extend back to the consummation of the previous endomictic period. So, it seems to be a more or less academic question whether rhythms and endomixis are independent. Certainly the rhythms occur for a while without endomixis in *sensu stricto*—but in all such cases the culture has died before very long.

This leads to the third point of interest which is the death of the cultures after the cessation of endomictic phenomena. This is true in each instance: *At*, *AEt*, and *Ot* (cf. Fig. 2). The data from these experiments perhaps are not sufficiently extensive to make sure that this is not a case of *post hoc sed non propter hoc*, but they make it highly probable that endomixis is necessary for the continued life of the race.

C.

The data which have been presented in the study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under varied and under constant culture conditions, may now be analyzed from the point of view of the periodicity of these phenomena in the *same* races under varied and under constant culture conditions, since experiments *A* and *B* of this series were conducted simultaneously.

This analysis is readily made by a study of Figs. 3, 4, 5 and 6, which consist of a combination of the graphs already presented from the other point of view. The graphs are paired, one above the other, so that identical five-day periods coincide. For example, in Fig. 3, period 10 of *A* is directly above the same period of *At*. Since the endomictic periods of all these cultures have just been considered, it is only necessary to tabulate them for reference:

A	underwent	endomixis	at	periods	....	4	9	15	21	28
At	"	"	"	"	....	4	9	0	0	0 died
AE	"	"	"	"	....	4	9	15	0 died	28
AEt	"	"	"	"	....	4	9	15	0	0 died
O	"	"	"	"	....	4	9	15	20 died	28
Ot	"	"	"	"	....	4	9	0	—	died
B	"	"	"	"	....				21	28
Bt	"	"	"	"	....				0	28

From this table it is apparent that the synchronism of endomixis is practically perfect in the *same* races when bred under *different* environmental conditions. The first two experiments showed that it was the same for *different* races when bred under the *same* environmental conditions whether varied or constant. Therefore this experiment corroborates and broadens the conclusions derived from the former ones and shows clearly

that the periodicity of endomixis is largely independent of the character of the culture medium—the general environmental conditions—within the rather wide limits in which it has been varied in parts *A*, *B* and *C* of this series of experiments. At most the culture conditions initially influence the appearance of endomixis. In other words, the organism is set, so to speak, to undergo endomixis approximately once a month and this it does under any more or less favorable environmental conditions. A sudden change, however, of these conditions may bring about endomixis slightly earlier than it otherwise would have occurred but after this the usual rhythmic period of the species is maintained.

Experiments—Series II.

The experiments of this series may be outlined as follows:

A. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *large* amount of culture medium supplied fresh *daily*.

B. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *small* amount of culture medium changed on *alternate days*.

C. Study of the periodicity of rhythms and endomixis in the *same* races of *Paramecium aurelia* when bred in a relatively *large* amount of culture medium supplied fresh *daily*, and in a relatively *small* amount of culture medium changed on *alternate days*.

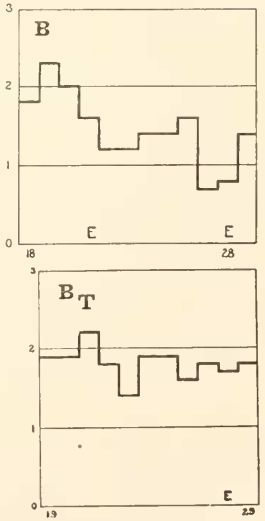

Fig. 6.

These experiments were begun on July 2, 1915, and continued until February 22, 1916. Five different races of the organism were employed. Three of them (*A*, *O*, and *B*) were the same subcultures which were used in the experiments of Series I., and which had been continued during the interim—that is, from March, 1915, to July, 1915. Therefore the numbering of the five-day periods was continued from the earlier work and the

first period of the present series is number 53. Two new races were secured from diverse localities as already described and subcultures from these were begun and made a part of this experiment at once. These subcultures were designated *M* and *W*.

All these subcultures were supplied daily with the regular varied culture medium. Sister subcultures (designated *As*, *Os*, *Bs* and *Ms*) were isolated line by line from the above-mentioned subcultures at the start of the work and were subjected to the same varied culture medium but the amount was reduced to one half that supplied to the parent subcultures and the medium was changed at intervals of 48 hours instead of 24 hours.

It has been shown in previous papers,[1] that the rate of reproduction of *Paramecium* is markedly influenced by the volume and the freshness of the culture medium. This was found to result from the accumulation of the excretion products, in view of the fact that a medium which contains the excretion products of a heavy growth of paramecia has a decidedly depressing effect on the division rate of this organism.

Accordingly it seemed that the excretion products of *Paramecium* afforded the most natural means of quickly modifying the division rate in order to determine the effect of this on the rhythms and endomictic periods. Obviously the experimental conditions involve two variables—excretion products, and their effect, the lowering of the fission rate—so that the specific influence of one or the other cannot be determined from the data given below. But that is not of importance in the present work which is merely an endeavor, as already stated, to determine the effect of normal environmental changes.

A.

This set of cultures was carried on in order to study the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *large* amount of varied culture medium supplied fresh *daily*. The experi-

[1] Woodruff, "The Effect of Excretion Products of *Paramecium* on its Rate of Reproduction," *Journ. Exper. Zoöl.*, X., 1911; Woodruff, "The Effect of Excretion Products of Infusoria on the Same and on Different Species, with Special Reference to the Protozoan Sequence in Infusions," *Journ. Exper. Zoöl.*, XIV., 1913.

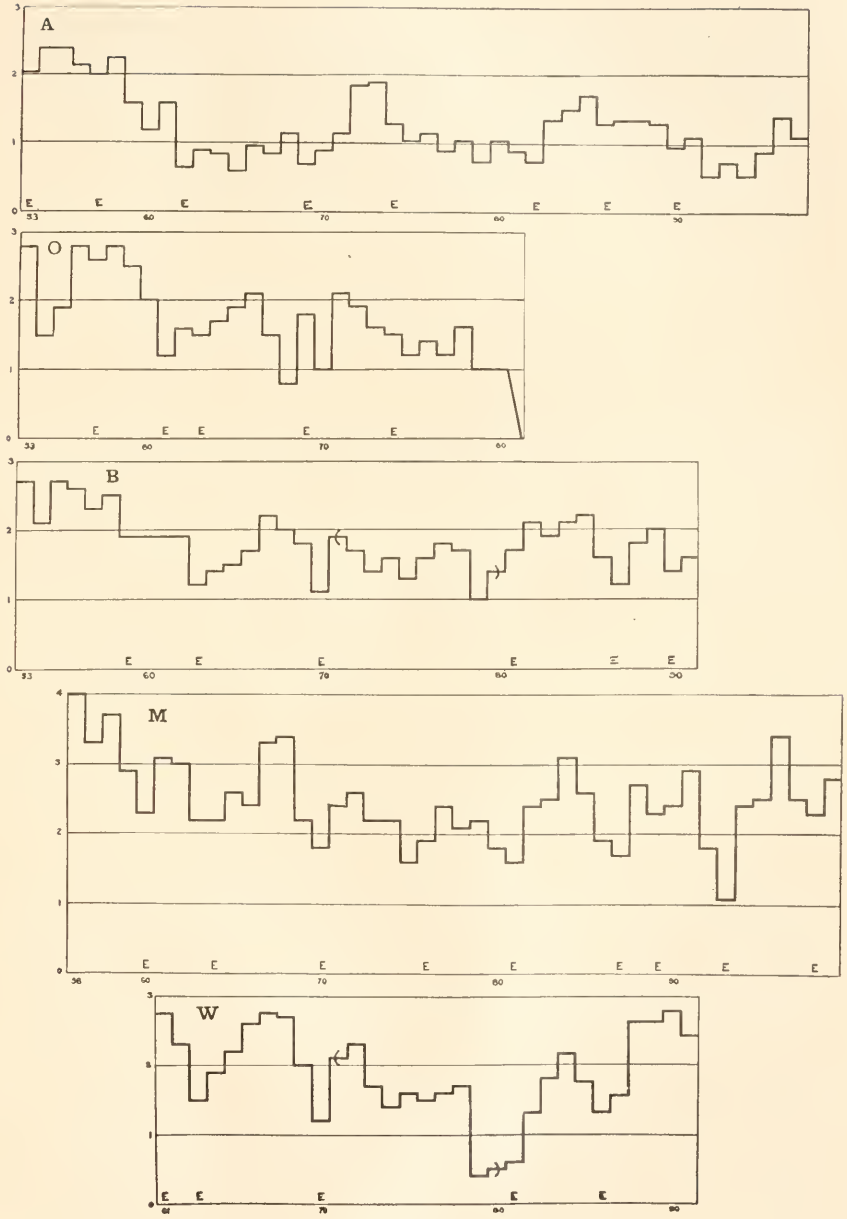

FIG. 7.

ment is essentially a repetition of experiment *A* of Series I (cf. p. 443) since three races (*A*, *O* and *B*) and the character and amount of culture medium are the same as employed before. The results, therefore, now will be analyzed from the viewpoint of Series I. and later from that of the present series.

The results are shown graphically in Fig. 7 from which it is evident that:

A	underwent	endomixis	at	periods	...	53	57	62	69	74	82	86	90		
O	"	"	"	"	...		57	61–63	69	74					
B	"	"	"	"	...		59	63	70	[1]	81	87	90		
M	"	"	"	"	...		60	64	70	76	81	87	89	93	98
W	"	"	"	"	...			61–63	70	[1]	81	86			

A study of this table and the graphs which it summarizes shows again practically the same periodicity of endomixis in diverse races on the varied culture medium as was observed in experiment *A* of Series I. The synchronism is not quite as exact as in the former experiment but, considering all the unknown and uncontrollable variables in such a long experiment, it clearly offers further support for the conclusion that rhythms and endomixis are essentially independent of environmental conditions, and that the culture conditions merely influence initially, if at all, the appearance of endomixis, and that once established the rhythmic period characteristic of the species is maintained within rather narrow limits.

B.

This set of experiments involves a study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *small* amount of culture medium changed on *alternate days*. The cultures used, as already stated, were *As*, *Os*, *Bs* and *Ms* and their behavior with respect to the process under consideration is given in Fig. 8. A study of this graph shows that endomixis was observed in:

As at periods	59	61	65	70	[1]	0	85	90
Os " "	59	0	64	69	[1]	82	86	
Bs " "	59	0	64	70	died			
Ms " "	59	62	64	70	[1]	82	87	91

[1] The animals were not studied cytologically during this period.

Again the synchronism is very marked throughout the work, while during the initial period (59) it is perfect. The explanation of the occurrence of endomixis in every culture during the 59th period is undoubtedly due to the *marked* change of culture conditions to which these subcultures were subjected, when the experiment was initiated in period 58, by isolation of the 's'

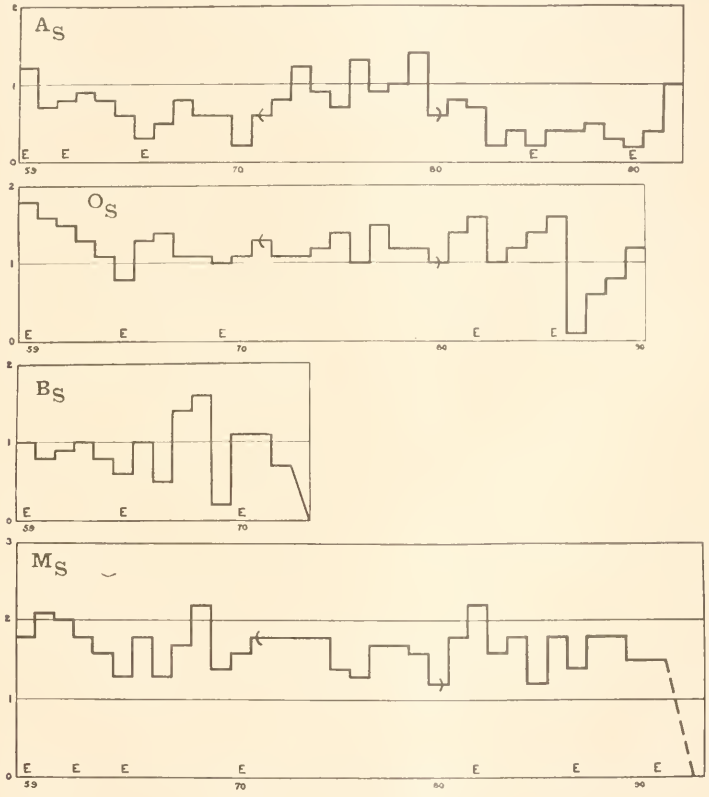

FIG. 8.

subcultures from their respective main cultures on the varied culture medium changed daily (cf. Figs. 9 to 12). This will be considered in connection with the following experiment, but obviously it is in accord with the results obtained by Woodruff and Erdmann[1] who noted in studying various *lines* of one race that the reorganization process "may occur a few days earlier in

[1] *Journal of Experimental Zoölogy*, 1914.

animals not supplied daily with fresh culture fluid than in the regular lines."

It is apparent then from this experiment that the same general conclusion which was derived from all the previous ones is again justified, viz., the culture conditions may, at most, initially influence the appearance of endomixis; but once established the rhythmic period characteristic of the species is maintained with great exactness, resulting in a remarkable synchronism of the process in the different races. General normal environmental changes obviously do not permanently alter the fundamental inherent rhythmic periods of the organism.

C.

A study now can be made of the phenomena under consideration in the *same* races of *Paramecium aurelia* when bred in a relatively large amount of culture medium supplied fresh daily, and in a relatively small amount of culture medium changed on alternate days. This involves, obviously, the comparison of the results from the two previous experiments since these were conducted simultaneously and afford the requisite data. Therefore the culture graphs of these two experiments are presented, one above the other, so that identical five-day periods coincide, in Figs. 9, 10, 11 and 12.

It is to be noted that *As* and *Os* were branched from *A* and *O* very soon after endomixis had occurred in the latter cultures; *Bs* came from *B* during the actual occurrence of endomixis; while *Ms* was branched from *M* toward the end of a rhythm as the subsequent appearance of the process in *M* at period 60 shows.

The four figures mentioned and the following tabulation of the periods in which endomixis occurred in the various subcultures shows the synchronism of the reorganization process in all the pairs of cultures under the markedly different environmental conditions. It is difficult to say whether this coincidence of the process is more exact between different races under the same culture conditions or between the same races under different culture conditions, because most of the variations are so small that they fall well within the limits of error involved in the five-day plotting method, etc. (cf. p. 440).

A at periods..	53	57	62	?	69	74	82	86	90
As " " ..		59	61	65	70	[1]	0	85	90
O " " ..		57	61	63	69	74	died		
Os " " ..		59	0	64	69	[1]	82	86	
B " " ..		59	0	63	70	[1]	81	87	90
Bs " " ..		59	0	64	70	died			
M " " ..			60	64	70	76	81	87	89
Ms " " ..		59	62	64	70	[1]	82	87	91

Thus clearly, in the long run, the *s* environment had no effect on the periodicity of the process. But, as pointed out before, endomixis appeared without exception in the four *s* cultures immediately upon their isolation from the respective parent cultures and on subjection to the stale culture fluid. Therefore, now that it is possible to compare parent and daughter lines, the obvious conclusion from the data is that endomixis was brought about earlier (except in *Bs* which was started during endomixis) by the changed cultural conditions; that is, earlier

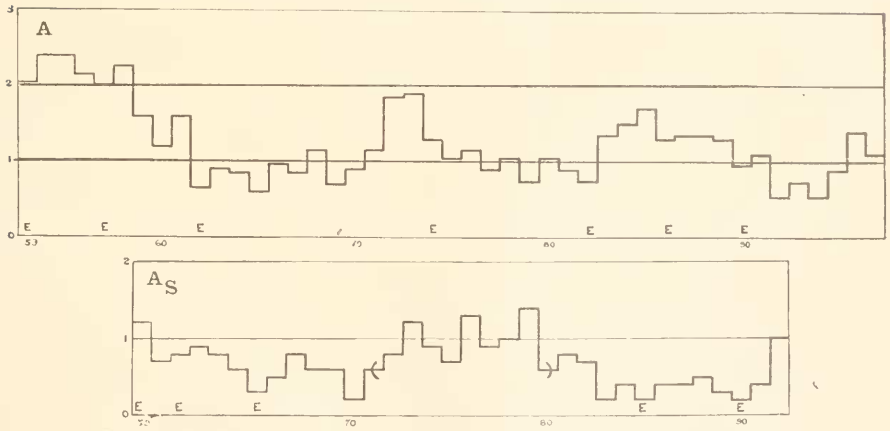

FIG. 9.

than it appeared in the parent cultures. This result, it may be noted, substantiates the conclusion from the former experiments that the remarkably exact synchronism of endomixis of various races under the most diverse environmental conditions is due to a slight initial influence on the occurrence of the process.

Figs. 9, 10, 11 and 12 show that the treatment to which the *s* cultures were subjected resulted in a distinctly lower rate of

[1] Not studied cytologically during this period.

division—on the average about three quarters of division per day lower than in the cultures subjected to the fresh culture medium, etc. This is without doubt due, as already discussed, in large part at least to the accumulated excretion products in the *s* series. But whatever the cause, the experiment affords an opportunity to study the effect of naturally changed conditions, involving a lowered fission rate, on the periodicity of rhythms and endomixis.

Now since the *s* subcultures divided at a much lower rate than the parent cultures, and since endomixis appeared fairly synchronously in parent and *s* sets, it is obvious that endomixis consistently appeared in the *s* subcultures within a smaller number of generations. In other words, the treatment of the *s*

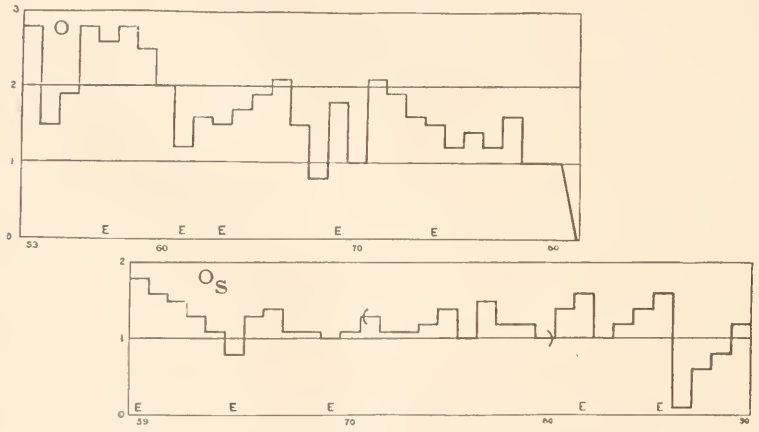

FIG. 10.

series apparently resulted merely in reducing the number of cell divisions in a given time and had practically no effect (except in the first period) on the occurrence of endomixis. On the basis of this set of cultures, then, endomixis is to a certain extent independent of the number of generations and more closely related to a time factor, if such an expression may be employed.

The *B* pair of cultures affords a fairly typical example (Fig. 11). In *B* endomixis occurred at periods 59, 63 and 70; while in *Bs* it occurred at periods 59, 64 and 70. Thus the length of

time in days between successive reorganizations is 20 and 35 in *B*, and 25 and 30 in *Bs*. Therefore in both cultures the same number of days (55) elapsed from one endomixis to its second following occurrence. On the other hand in *B* the process was in progress at the 335th, 370th and 435th generations, that is at intervals of 35 and 65 generations, while in *Bs* it took place at the 335th (when *Bs* was isolated from *B*), 360th and 380th genera-

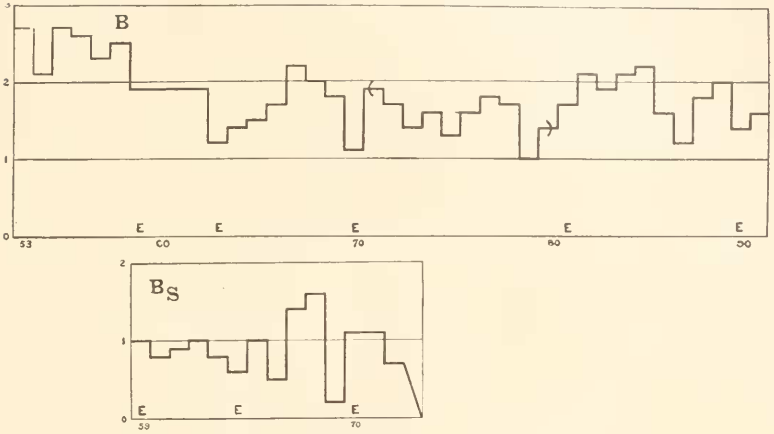

FIG. 11.

tions, or at intervals of 25 and 20 generations. Therefore in *B* 100 generations were attained from one endomictic period to the second following occurrence of the process; while in *Bs* only 45 generations occurred during the same period. To repeat: the experimental treatment apparently simply reduced the number of generations during the 100 days.

This same general result was obtained in Set *C* of Series I., though of course here the lowering of the division rate was the result of other causes. Figs. 3 and 4 show that endomixis occurred in the fourth period of the four subcultures, *A* and *At* and *AE* and *AEt*, although the rate of division and therefore the number of generations was less in *At* than in *A*, and less in *AEt* than in *AE*.

This set of experiments, then, corroborates in a clear-cut manner the general result derived from all the previous ones; that is, general environmental changes, especially if they are

pronounced, usually bring on endomixis slightly earlier than it would have occurred if the cells had been left in the environment in which they were at the last reorganization period. But after this initial change the periodicity characteristic of the organism is resumed and persists.

In addition, however, this experiment suggests another point of interest: the length of the rhythm is apparently partially

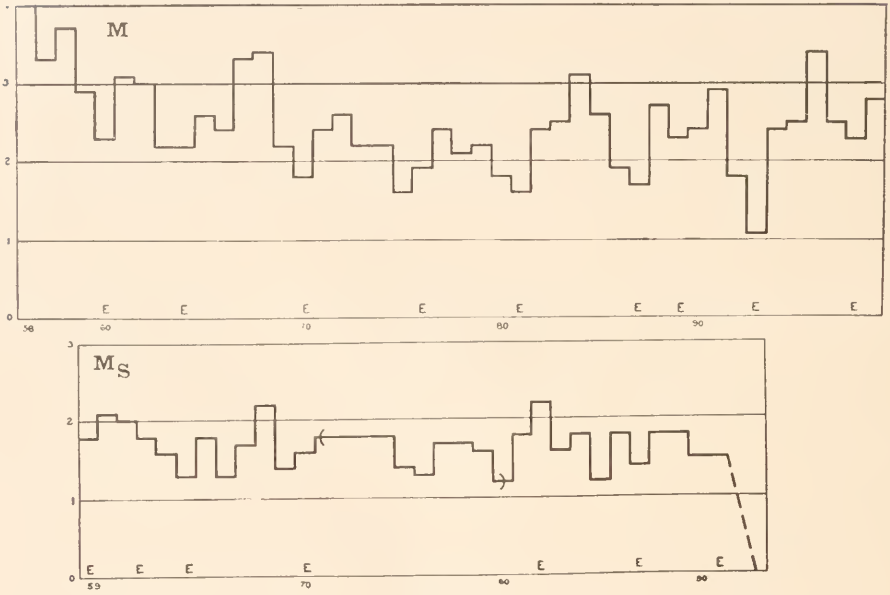

FIG. 12.

independent of the number of cell divisions—the periodicity being one in which time, so to speak, is an important factor. This is a most surprising result, because such profound reorganization phenomena as are involved in endomixis must bear a more or less definite relation to the physiological activity of the protoplasm, the best criterion of which is generally considered to be growth and reproduction as indicated by the division rate. More experiments obviously are needed to resolve this 'time factor' into its significant elements.

EXPERIMENTS—SERIES III.

Peebles states that Horlick's Malted Milk is a most satisfactory culture medium for *Paramecium* if used in a .2 per cent.

solution,[1] and therefore this seemed to offer an opportunity to study the effect of a medium radically different in composition from those previously employed in these studies. Accordingly subcultures designated *Am*1, *Am*2, *Am*3, *AEm*1, *AEm*2, *Om* and *Mm* were started from *A*, *AE*, *O* and *M* respectively, and bred on this medium. The extent of these cultures and the time of appearance of endomixis (*E*) in the two sets is shown in the following table:

Period.....	52	53	54	55	56	57	58	59	60
A.....		*E*				*E*			
*Am*1.....	(from *A*)	*E*				*E*			
*Am*2.....	(" *A*)	*E*				*E*			
*Am*3.....						(from *A*)	*E*		
AE.....					*E*			*E*	
*AEm*1.....	(from *AE*)	*E*			*E*		discontinued		
*AEm*2.....						(from *AE*)	*E*		
O.....						*E*			
Om.....	(from *O*)					*E*			
M.....									*E*
Mm.....						(from *M*)	*E*		

This table shows that the malted milk medium did not change at all the periodicity of endomixis in either the *A* or *O* milk subcultures, while it consistently brought it about earlier in the *AE* and *M* milk series. The results from the *AE* and *M* cultures are therefore in accord with the results obtained in Series II of these experiments, while those with *A* and *O* apparently differ. However, the non-appearance of endomixis earlier in the *Am* series is readily explained by the fact that the process was just about to occur in the parent culture when the *Am* subcultures were branched, as is shown by the fact that it appeared in *A* during the following period.

The experiments with malted milk are obviously not extensive enough to give any details of the effect of long subjection to this medium on endomictic periodicity, but they adequately answer, it is believed, the purpose of the present study by again indicating that the appearance of endomixis may be slightly

[1] Peebles, "Regeneration and Regulation in *Paramecium caudatum*," BIOL. BULL., 1912.

altered temporarily by subjecting *Paramecium* to a markedly changed environment.

Experiments—Series IV.

This series is a brief repetition of Series I., since it comprises a comparison of the periodicity of endomixis both in the same races when bred under varied and constant culture conditions, and in different races bred under varied and constant culture conditions. The cultures employed were *A*, *AE* and *B* which were used in the first series, and also two other cultures, *M* and *W*.

The following table shows a practically perfect synchronism of endomixis in all the races under the different environmental conditions:

Period.....	81	82	83	86	87	88	89	90
A.....		*E*		*E*				*E*
At2.....			(from *A*)	*E*				*E*
AE.....		*E*		*E*				*E*
AEt2.....			(from *AE*)	*E*				*E*
B.....	*E*				*E*			*E*
Bt2.....			(from *B*)	*E*				*E*
M.....	*E*				*E*		*E*	
Mt2.....			(from *M*)	*E*				*E*
W.....	*E*			*E*				
Wt2.....			(from *W*)	*E*				

This is exactly the same result which was obtained in the experiments of Series I. In Series I this synchronism was most satisfactorily accounted for by assuming that there was an initial effect on the periodicity by the change of environmental conditions to which all the cultures were subjected at the start. That this assumption was justified is shown by experiment *C*, Series II (cf. p. 455). However, it is to be noted in the present experiment that there is no apparent initial influence of the changed culture conditions, but this is probably due to the fact that the *A*, *AE*, *B*, *M* and *W* cultures had been so long under the same environmental conditions before their respective *t2* subcultures were branched from them. Consequently the syn-

chronism of the *t2* set is due merely to maintaining the periodicity of the respective parent cultures.

General Summary.

All four series of experiments show that the general 'time-periodicity' of rhythms and endomixis in *Paramecium aurelia* is the same in the several races which have been studied under the following environmental conditions:

1. Varied culture medium changed daily, and at room temperatures.
2. Varied culture medium changed on alternate days, and at room temperatures.
3. Constant beef extract culture medium and at a temperature of 26° C.
4. Horlick's malted milk medium, and at room temperatures.

Thus it seems clear that one question which this study was planned to elucidate has been answered: General changes in the environment of the animals, as markedly different culture media and temperatures, such as may be termed normal changes, do not permanently modify the length of the rhythm or the time between successive endomictic periods which is characteristic of the species.

However, sudden marked changes in normal culture conditions may initially induce the appearance of the definitive endomictic phenomena slightly earlier than they would have occurred if the cell had been continued under its former environmental conditions; but this initial disturbance is soon compensated for, usually within the present rhythm, so that the previous characteristic periodicity is again resumed.

Throughout all the work there is evident a remarkable *synchronism* of the endomictic process in all the races bred simultaneously, regardless of the environmental conditions. Thus not only is the periodicity of endomixis, or length of the rhythm, the same, as stated above, but also the rhythmic periods are synchronous. The explanation of this is clearly due, in the experiments involving the most marked changes in the cultural conditions, to an initial effect of these changes, which brings into line, so to speak, the appearance of endomixis in all the cultures.

Consequently it is highly probable that a slight initial shift of the definitive onset of endomixis in the various races is the explanation of the nearly simultaneous appearance of the process in all the races under all the conditions.

Although the 'time-periodicity' characteristic of the species has been shown by the present experiments to be practically unmodifiable under the general environmental changes which were employed, it has been found that the 'generation-periodicity'—or the number of cell divisions between one occurrence of endomixis and the next—may be modified to a considerable degree by the culture conditions which lower the division rate. In other words, the rhythm appears to be more susceptible of modification in regard to generations than time. As has been previously noted, this is a surprising result, since a profound reorganization process such as endomixis must be closely related to the general metabolism of the cell and this is expressed to a large extent in growth and reproduction. Further work on this problem is in progress.

Finally, the cessation of endomixis in these experiments was invariably followed, usually within a rhythm or two, by the death of the culture involved. This indicates strongly, if it does not prove, that a periodic occurrence of the definitive endomictic phenomena is a *sine qua non* for the continued life of the race—a conclusion which is concordant with all previous data in regard to endomixis.